ProHeart 6 (moxidectin)

Fort Dodge Animal Health
A Division of Wyeth

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EXECUTIVE SUMMARY

Canine heartworm infection (*Dirofilaria immitis)* occurs in many parts of the world, including all 50 states in the US. Despite widespread availability of monthly heartworm preventatives, the infection rate in the US increased in the 1990s, and use of heartworm preventatives declined. Surveys have shown that compliance (i.e., reliable monthly treatments by owners) is problematical and a limiting factor in the control of heartworm in the dog population.

The active ingredient in ProHeart® 6 is moxidectin, a macrocyclic lactone. Moxidectin has been thoroughly evaluated to determine its toxicological and metabolic properties in multiple animal species and is approved as an anthelmintic agent for use in cattle, sheep, swine, dogs,and horses, in 70 countries. ProHeart 6 has been carefully evaluated for the treatment of heartworms and hookworms in dogs. In registration studies sponsored by Fort Dodge Animal Health (FDAH) (a Division of Wyeth), the product has been demonstrated to be safe and efficacious. Further, it appears to be well tolerated in canine breeds that do not tolerate some of the monthly products.

In June 2001, Proheart 6 (moxidectin) was approved and launched in the US by FDAH to prevent canine heartworm disease for 6 months, and to treat existing larval and adult stages of the canine hookworm. Since then, the product has also been registered in Italy, Canada, Japan, France, Greece, Portugal, Spain, and Korea. A similar product, ProHeart SR12, which contains approximately 3 times the amount of moxidectin as ProHeart 6, is registered and marketed in Australia since October 2000.

Among heartworm preventative medications for dogs, ProHeart 6 is an innovative product. Unlike conventional products that require monthly dosing for a minimum of 1 month prior to mosquito exposure through 1 month after mosquito exposure, in order to achieve protection against heartworm infection over a mosquito-infestation season, 1 injection of ProHeart 6 (moxidectin) sustained-release product provides 6 months of protection. ProHeart 6 was specifically developed to overcome field efficacy problems that result from poor compliance with monthly treatments. The introduction of ProHeart 6 provided an avenue for continuous protection against heartworm infection.

Shortly after launch of ProHeart 6, FDAH received a number of reports of allergic-type reactions after administration. The reactions ranged from mild and self-limiting to severe anaphylactoid reactions. Product analysis of minor component profiles between batches of ProHeart 6 revealed a trend to lower reactions for lots with no detectable residual solvents. At this time, FDAH was continuing to optimize the manufacturing process. As part of this process, the decision was made that all ProHeart products would be produced from moxidectin technical material with no detectable solvents. After the manufacturing change was implemented, there was a decline in the adverse-event reporting rate from all markets.

Since the launch of the product, FDA expressed concern about the number and seriousness of adverse event reports (AERs). The vast majority of the AERs were submitted by FDAH based on field reports from veterinarians and dog owners. The reports were submitted under mandatory reporting regulations without assessment of the likelihood of association of the AER with Proheart 6 administration. As a result of FDA's concern, FDAH has made revisions to the product label and issued "Dear Doctor" letters. On September 3, 2004, based on continued FDA concerns, FDAH agreed to voluntarily recall the product from the U.S. market. The recall prompted regulatory authorities in Canada, Australia, Japan and Europe to further review the safety of ProHeart 6. These authorities have allowed continued marketing of all FDAH moxidectin products for canine heartworm control.

FDAH's postmarketing surveillance and analysis of AERs from June 2001 through August 2004 show the number of these AERs were generally decreasing. The peak of AERs in the second quarter of each year corresponds to peak-use periods and also appear to be decreasing over time. Analysis of AERs for ProHeart 6 by category show that the occurrence of injection-site reports remained low and consistent with other injectable products in the FDAH database. Allergy AERs trended down over time at 1.26 per 10,000 doses. Non-allergy AERs were low and consistent over time at 1.19 per 10,000 doses (neurologic at 0.12 per 10,000 doses; hematologic at 0.09 per 10,000 doses; hepatic 0.07 per 10,000 doses; cardiac at 0.02 per 10,000 doses; neoplasia at 0.06 per 10,000 doses). When taken in context with usage, the overall rate for AERs was low and trending down over time, up to the point of the recall.

FDAH recently sponsored a study of the safety of ProHeart 6 use in general veterinary practice and a comparison to the monthly oral products. The study utilized a database covering 403 full-service veterinary hospitals in 42 states and did not rely on voluntary reporting. The review evaluated approximately 7 million canine office visits with an emphasis on comparison of heartworm product safety with and without concomitant vaccine administration. Overall, the safety profile of ProHeart was similar to that of 2 commonly used monthly oral products. Many of the adverse events could be attributed to concomitant vaccine administration. The results of the study provide no support for the withdrawal of ProHeart 6 from the market.

In conclusion, FDAH has performed additional research and further evaluation of the ProHeart 6 database to add to the extensive safety and toxicology database for moxidectin and formulated products. These evaluations provide additional support for the safety and efficacy of the product and were conducted to address questions raised by FDA.

1.0 INTRODUCTION

Moxidectin is a semi-synthetic methoxime derivative of nemadectin that is a fermentation product of *Streptomyces cyaneogriseus* subspecies *noncyanogenus*. It is a pentacyclic 16-membered lactone macrolide. Moxidectin is licensed and marketed worldwide by Fort Dodge Animal Health (FDAH) (a Division of Wyeth) as an anthelmintic agent that causes the paralysis and death of affected parasites in cattle, sheep, swine, horses, and dogs. It is currently being developed in a collaboration between Wyeth and the World Health Organization (WHO) for humans with onchocerciasis (river blindness), a disease caused by infection with the tissue filarial nematode *Onchocerca volvulus.*

ProHeart® 6 (moxidectin) was approved in the US and launched by FDAH in June 2001, to prevent canine heartworm (*Dirofilaria immitis)* disease for 6 months, and to treat existing larval and adult stages of the canine hookworms (*Ancylostoma caninum* and *Uncinaria stenocephala*). Since then, this product has also been registered in Italy, Canada, Japan, France, Greece, Portugal, Spain, and Korea. A similar product, ProHeart® SR 12, which contains approximately 3 times the amount of moxidectin as ProHeart 6 and provides 12 months of protection is registered and marketed in Australia since October 2000.

Among heartworm preventative medications for dogs, ProHeart 6 is an innovative product. Unlike conventional oral tablets or topical applications that require monthly dosing for a minimum of 1 month prior to mosquito exposure through 1 month after mosquito exposure, in order to achieve protection against heartworm infection over a mosquito-infestation season, 1 subcutaneous (SC) injection of ProHeart 6 (moxidectin) sustained-release product provides sustained 6-month protection. The single administration of ProHeart 6 for a 6-month period of protection eliminates the possibility of the pet owner missing 1 or more monthly doses, which is the primary cause of lack of efficacy associated with these heartworm medications.

ProHeart 6 has been well received by veterinary professionals and dog owners as evidenced by its increasing market share in major markets since launch. By the third quarter of 2004, ProHeart 6 was the number two product in the US with a 24% market share. In Italy, it is expected to

achieve a 35% share by the end of 2004. ProHeart SR 12 is the market leader in Australia, presently with a 47% share.

Subsequent to the product approval in June 2001, the FDA Center for Veterinary Medicine (CVM) raised concerns over the number of reports of adverse events associated with ProHeart 6.[1] Further concern was raised that "many of the reports received have involved serious, life-threatening adverse events such as anaphylaxis, convulsions, hematopoetic disorders, hepatopathies" and also "neurologic problems and unusual cardiac signs." It was also stated that "Pet owners should be advised on appropriate alternative heartworm preventatives for their dogs." FDA requested "that the firm continue to conduct research to determine the cause of related adverse reactions…before the product is marketed again."

On September 3, 2004, FDAH announced that it was voluntarily ceasing production for the US market and recalling ProHeart 6 from the US market until resolution of FDA safety concerns, based on reports of adverse events. Despite the voluntary recall, FDAH maintains that ProHeart 6 is safe and efficacious with acceptable field performance. Regulatory authorities in Canada, Australia, Japan, and Europe have allowed continued marketing of the FDAH moxidectin products for heartworm. FDAH supports the FDA formation of an independent Advisory Panel to review safety data on ProHeart 6. In order to provide the Advisory Panel with extensive analyses of available scientific data on ProHeart 6, FDAH sought assistance from independent experts. As requested by the FDA, additional research has been conducted to better define the adverse reactions. FDAH anticipates that comprehensive review of available ProHeart 6 data by the Advisory Panel will satisfactorily resolve FDA safety concerns.

Careful evaluation of the nature and timing of the AERs identified that allergy-based signs could be attributed to treatment and occurred shortly after treatment. The true incidence of allergic events to ProHeart6 is confounded because many dogs receive concurrent vaccinations. These are recognized to trigger allergic manifestations. A range of commonly occurring disease conditions that affect dogs is also seen in the AERs. These appear to represent baseline occurrence of these conditions in the canine population.

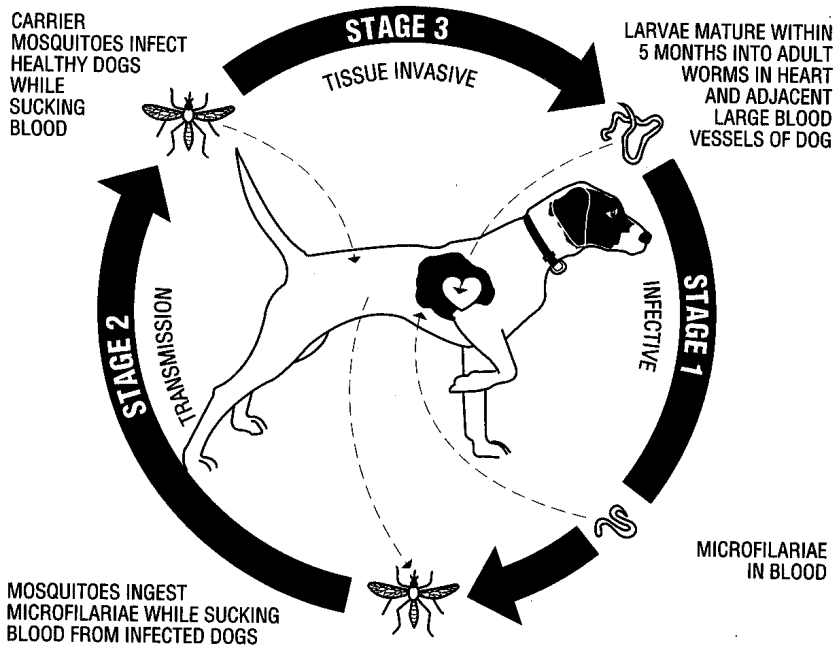
The purpose of this document is to present an overview of moxidectin and ProHeart 6 information from non-clinical studies, clinical trials, adverse event reporting, and analysis of safety and efficacy data for review by the Advisory Panel. This information includes new data on pharmacokinetics, new pharmacological investigations, new evaluation of adverse event findings, and epidemiological information from 7.0 million canine visits to veterinary clinics. FDAH is confident that the new research and re-evaluation of previous data support the safety and efficacy of ProHeart 6 and looks forward to returning this product to the market.

2.0 REVIEW OF HEARTWORM IN DOGS

2.1 Life Cycle of Dog Heartworm (*Dirofilaria immitis*)

The life cycle of the dog heartworm can be described in 3 stages as shown.

Figure 2.1-1. Life Cycle of Dog Heartworm



Stage 1: Adult female worms release tiny immature heartworms called microfilariae into the bloodstream of the infected dog. Adult worms are reported to live for 2 to 7 years.

Stage 2: The infection is spread from dog to dog by mosquitoes. When a mosquito bites an infected dog and feeds on blood, it takes in some of these microfilariae. Within 2 to 3 weeks, these microfilariae will develop into a stage which can infect other dogs when they are bitten by the same mosquito as it feeds.[2,3]

Stage 3: Following infection, the immature stages grow and develop over 2 to 3 months in the subcutaneous (SC) tissues, muscle, and fatty tissues of the dog. They then migrate, and from 70 to 120 days post infection, stages of heartworm may be found in the heart or pulmonary artery. These develop into adult heartworms which are long slender roundworms, normally living in the right side of the heart and nearby blood vessels. The worms may be up to 35 cm in length when mature.[2,3]

Mature female *Dirofilaria immitis* begin releasing microfilariae into the blood stream some 6 to 7 months after infection, thus completing the lifecycle.

Heartworm preventative products exert their effect at Stage 3, the tissue migrating stage.

Heartworm tests rely on detection of the parasite either at Stage 2, by detection of circulating microfilariae, or at Stage 3, by detection of female heartworm antigen. Heartworm antigen tests are not positive until the infection is at least 5 months old, they are inconsistently positive at 5 to 7 months, and are not considered to be reliably diagnostic until the infection (with female worms present) is at least 8 months old. These tests are highly sensitive and specific. If clinical signs raise any doubt about the accuracy of the particular test result, the test should be repeated, preferably with a different test kit or laboratory.[4]

2.2 Heartworm Disease in Dogs

Many dogs may be infected for several months with *Dirofilaria immitis* without showing clinical signs. This is particularly true for inactive dogs. However, immature worms in the pulmonary artery can initiate disease as early as 3 months after infection.[5] Adult worms in the heart cause inflammation of the heart lining and valves, and can eventually lead to heart failure. The effect on blood flow can lead to problems in other organs, particularly lungs and kidneys.

It can be difficult to know that a dog is affected in all stages of the disease because symptoms may be slight. The dog may only be listless and tire easily. Performance in working dogs may be affected. As the disease progresses, there may be cough, loss of condition, and build-up of fluid in the abdomen. Severely affected dogs may die.

Treatment of the disease can be risky, and early detection before chronic damage occurs to heart and lungs is important. This is because thromboembolic complications may occur, particularly in heavily infected dogs with pulmonary arterial vascular obstruction, and especially if congestive heart failure is present. The approved adulticide treatment is melarsomine, trade name Immiticide, an arsenical compound. While melarsomine is less toxic and more effective than its predecessor, thiacetarsamide, it is administered as a deep intramuscular injection into the lumbar muscles to reduce swelling and soreness at the injection site. Pulmonary thrombo-embolism and/or shock may occur following adulticide treatment even in symptomless dogs. Exercise restriction during the recovery period (weeks) is essential to minimize cardiopulmonary complications. Off label use of ivermectin for this purpose has been reported, but is not recommended because of progression of heartworm disease in the treated dogs.[6]

2.3 Canine Heartworm Epidemiology in the US

Heartworm infection in dogs has been diagnosed in many parts of the world, including all 50 states of the United States. While heartworm is considered endemic in the 48 contiguous states and Hawaii, transmission has not been documented in Alaska, even though there has been importation of infected dogs. It is likely that the climate is not conducive to the maturation of infective larvae. Adequate temperature and humidity are required both to support a suitable mosquito population and also to provide sufficient heat for maturation to the infective larval stage (L_3). Laboratory studies indicate that at 80°F, 10 to 14 days are required for maturation to infective stage, this period is longer at lower temperatures or where significant diurnal temperature fluctuation occurs.[2,3]

Therefore, the length of the heartworm transmission season varies with geographical location and climatic factors. The peak months for heartworm transmission in the northern hemisphere are July and August. Estimates of the duration of the transmission season vary from less than 4 months in Southern Canada to essentially all year in sub-tropical areas such as Florida and the Gulf Coast. It is believed that transmission occurs for 6 months or less above the 37th parallel.

The prevalence of heartworm in dogs varies from state to state, with higher infection rates in southern states, and particularly higher along the Mississippi and Ohio rivers. The number of adult heartworms present in infected dogs has been reported to be lower in the North (Michigan) than in the South.[7] A survey undertaken by the American Heartworm Society in 2001, published

in 2002, reported the highest infection rates in Texas, followed by Florida, Louisiana, North Carolina, Georgia, Mississippi, Tennessee, South Carolina, Alabama, and Indiana. Of significant veterinary concern, the survey reported that despite the widespread availability of monthly heartworm preventatives, the rates of infection with heartworm had not decreased over the previous 10 years.[2,8,9] For further information see Section 4.5.

For the past 2 decades, heartworm prevention relied on monthly administration of macrocyclic lactones, sometimes in combination with other active ingredients to treat other parasites. Commonly used products include ivermectin, milbemycin oxime, and selamectin. Combination products include ivermectin plus pyrantel (for roundworm control) and generics, and milbemycin oxime plus lufenuron (for flea control). For dogs that suffered toxic effects from these compounds, the only alternative available was daily treatment with diethyl carbamazine.

The introduction of ProHeart 6 in the US in the second half of 2001 provided 6 months of continuous protection against heartworm infection without having to rely on monthly treatment by the dogs' owners, a major source of lack of efficacy.

3.0 MOXIDECTIN OVERVIEW

3.1 Pharmacology – Mechanism of Action

Moxidectin has been shown to have activity at the γ-aminobutyric acid (GABA)-A receptor-chloride channel complex resulting in an influx of chloride ions and hyperpolarization of cell membranes. Hyperpolarization causes the nerve fibers to be less excitatory and results in paralysis and death of the parasitic organism. Another proposed mechanism of action for moxidectin is through activity at glutamate-gated chloride ion channels. The specificity of moxidectin for the parasite versus the mammalian host results from 1) a low affinity for mammalian GABA-gated chloride channels, and 2) GABA-containing neurons and receptors are found in mammals in the central nervous system, whereas in arthropods and nematodes these are found in the neuromuscular junctions of the peripheral nervous system and thus are more accessible to a blood-borne therapeutic.

In dogs, the approved oral monthly dosage of moxidectin for prevention of heartworm is 3 µg/kg; the approved SC dosage of moxidectin as ProHeart 6 is 0.17 mg/kg administered every 6 months. In this and the sections that follow, dosages of ProHeart 6 or moxidectin expressed as mg/kg refer to mg of moxidectin per kg of animal body weight.

3.2 Pharmacokinetics and Drug Metabolism

Studies were conducted in various animal species to characterize the absorption, distribution, metabolism, and excretion of moxidectin after oral administration. Moxidectin was moderately absorbed with a bioavailability of 19% in rats, and had a long serum half-life of 23 to 45 hours in rats and 8.1 days in dogs after oral gavage administration. A single SC injection of the approved dosage of 0.17 mg/kg as ProHeart 6 to beagle dogs resulted in peak concentration in serum (C_{max}) of 5.1 ng/mL, a time to peak concentration (t_{max}) of 7 to10 days, an area under the concentration-versus-time curve ($AUC_{0-\infty}$) of 217 ng•days/mL, and an apparent elimination half-life ($t_{1/2}$) of approximately 35 days. After SC injection of ProHeart 6 once every six months for a total of 6 injections, there was no evidence of alterations in pharmacokinetic parameters or indication of accumulation. A recent study of the single administration of moxidectin in the diet to female dogs at 45 ppm (corresponding to approximately 1 mg/kg used in the 1-year toxicology study) resulted in a C_{max}, $AUC_{0-\infty}$, and $t_{1/2}$ values of 290 ng/mL, 678 ng•day/mL and 8.3 days, respectively. These recent studies in the dog, and an ongoing 28-day pharmacokinetic study in rats were designed to further evaluate the systemic exposure to moxidectin under the conditions of the previously conducted toxicology studies.

After oral administration, the major site of moxidectin distribution was in fat in rats, cattle, sheep, and horses. It was eliminated largely unmetabolized in the feces. The $t_{1/2}$ in fat in the rat was 11.5 days, much longer than in serum. Limited metabolism was noted in all species and minor metabolites were identified as predominantly mono- and di-hydroxylated moxidectin.

Moxidectin did not result in significant in vitro inhibition of cytochrome P450 isozymes, indicating that drug-drug interactions mediated through cytochrome P450 are unlikely to occur.

P-glycoproteins (P-gps) are transmembrane proteins that transport a wide variety of endogenous and exogenous molecules across cell membranes. Moxidectin, similar to other macrocyclic lactones, is a substrate for P-gps. This relationship is of clinical importance in the development of nematode resistance to ivermectin and plays a significant role in breed sensitivity. A mutation in the P-gp gene of ivermectin-sensitive Collie dogs has been shown to be responsible for ivermectin-induced CNS toxicity. Moxidectin, however, was well-tolerated by these ivermectin-sensitive dogs (see Section 4.1.3.2). Thus, moxidectin transport is less dependent on P-gp and subsequent toxicity is less likely manifest due to factors which alter P-gp activity.[10]

3.3 Toxicology

The toxicologic profile of moxidectin administered by the oral route has been well established. This profile is relevant to other routes of administration because of limited metabolism of moxidectin in the body and the long terminal half-life regardless of the route of administration. A more detailed discussion of the pharmacokinetics and toxicity assessment of moxidectin is presented in Appendix 6.1.

3.3.1 In Vitro Side-Effect Profiling

Moxidectin and moxidectin microspheres (as present in ProHeart 6) were recently tested in vitro for binding activity at 64 different biological receptors. This assay is commonly used in drug discovery and development to identify any ancillary pharmacologic activities of a molecule which may result in undesirable biological effects. A final concentration of 10 ng/mL moxidectin was tested, which is approximately two-fold the average C_{max} value in serum of dogs after an SC injection of ProHeart 6 at the clinical dosage of 0.17 mg/kg. The receptors tested included those for neurotransmitters and neurotransmitter-related receptors, ion channels, steroids, second messengers, prostaglandins, growth factors/hormones, brain/gut peptides and enzymes. Moxidectin in either form did not significantly inhibit the binding of appropriate

radioligands to these receptors, indicating a lack of significant, competitive binding activity for moxidectin at the concentration evaluated.. These results are consistent with the absence of undesirable pharmacologic and toxic effects of moxidectin in animal studies at plasma levels greater than those required for efficacy.

3.3.2 Single- and Repeat-Dose Toxicology Studies

Single-dose toxicology studies of moxidectin were conducted to assess effects after a single administration of large doses in the event of accidental overdose and to assist in selection of dose levels for subsequent repeat-dose toxicology studies. In single-dose toxicity studies of mice and rats given moxidectin orally, the median lethal dosage (LD_{50}) values were 118 mg/kg and 42 to 78 mg/kg in male and female mice, respectively, and 122 and 97 mg/kg in male and female rats, respectively. After a single SC dose, LD_{50} values were 285 and 247 mg/kg in male and female mice, respectively, and > 640 mg/kg in rats. Common clinical signs in these studies were decreased activity, tremors, and prostration. These studies demonstrate a large margin of safety for ProHeart 6 since the lethal SC dose in rats is more than 3700-fold the approved ProHeart 6 dose of 0.17 mg/kg.

Repeat-dose oral (diet) toxicity was evaluated to assess long-term consequences of repeated, daily oral exposure to moxidectin. The objective of these studies was to expose animals to high levels of moxidectin to identify potential toxic effects, and to include lower doses to assess a possible dose-response relationship and a dose without significant adverse effects (NOAEL).

The following studies were conducted: 4-week studies in mice, rats, and dogs; 13-week studies in rats and dogs; and a 1-year study in dogs. Evaluations consisted of mortality, clinical observations, body weight, food consumption, hematology, clinical chemistry (except mice), organ weights, and macroscopic and microscopic examinations of organs and tissues. Ophthalmic examinations and urinalysis were also included in the dog studies.

In repeat-dose diet toxicity studies in rats and dogs of durations up to 2 years in mice and rats, and 1-year in dogs, no target organs of toxicity were identified. There were no significant adverse histologic or biochemical effects to any organ system. There were no proliferative lesions identified in any tissue which may signal the development of neoplasia, and no increase in tumors in 2-year studies in mice or rats. The toxicity of moxidectin manifested at high doses in clinical signs such as lethargy, ataxia, tremors and mortality (rodents only) with concomitant decreases in food consumption and body weight. Such clinical signs of hypoexcitation are consistent with an exaggerated pharmacologic effect of moxidectin mediated via the GABA-receptor. Interim analysis at day 21 in an ongoing 28-day diet pharmacokinetic study of moxidectin in dogs at a concentration of 45 ppm in feed (approximately 1 mg/kg, the NOAEL in the 1-year dog toxicity study) revealed a serum concentration of 278.5 ng/mL 24 hours after the preceding dose in feed (ie, trough level). This value is approximately 234-fold the $AUC_{0-\infty}$ (217 ng•days/mL) observed for moxidectin after a single SC dose in dogs of ProHeart 6.

3.3.3 Carcinogenicity Studies

3.3.3.1 Mice

A 2-year carcinogenicity study was conducted in male and female mice at diet doses of 15, 30, and 60 ppm (lowered to 50 ppm due to high mortality at week 9). Mortality was increased in females at doses of 60/50 ppm during the last 13 weeks of the study. There were no compound-related findings in hematology values, organ weights, or at macroscopic or microscopic examination. There was no evidence of moxidectin-related target-organ toxicity or tumorigenicity.

3.3.3.2 Rats

A 2-year carcinogenicity study was conducted in male and female rats at diet doses of 15, 60 and 120 ppm (lowered to 100 ppm due to high mortality in females at week 8). There were no compound-related findings for hematology values, organ weights or at macroscopic or microscopic examination; there was no evidence of moxidectin-related target-organ toxicity or tumorigenicity.

3.3.4 Reproductive and Developmental Toxicity Studies

Reproductive toxicity was evaluated in a rat multigeneration diet study and in developmental studies in rats and rabbits dosed daily by oral gavage. Based on the results from these studies, moxidectin was not found to be a selective reproductive toxin in rats, nor a teratogen in rats or rabbits.

3.3.5 Genotoxicity Studies

Moxidectin was tested for genotoxicity in 4 in vitro and 2 in vivo standard test systems. These assays assessed the ability of moxidectin to induce gene mutations, chromosome damage, or increased DNA repair which may be related to the carcinogenic potential of the test article. Moxidectin was uniformly negative in these assays, indicating that moxidectin is not a genotoxic compound.

3.4 Experience with Oral Moxidectin in Human Volunteers

A study in healthy, male volunteers was conducted to assess the pharmacokinetics and safety of moxidectin given orally as part of the development of this compound for onchocerciasis therapy in humans. Safety assessments indicated that moxidectin was safe and well tolerated, with a slightly higher incidence of transient, mild, and moderate CNS adverse events (dizziness and somnolence) as compared to placebo. Moxidectin was safe and well tolerated in humans after single oral doses of 3 mg to 36 mg, the highest dose evaluated.

3.5 Conclusions

Moxidectin is a potent antiparasitic therapeutic that acts to paralyze susceptible organisms through activity at GABA- and glutamate-gated chloride ion channels. Moxidectin has a long half-life, distributes predominantly to fat, shows little metabolism, and is excreted primarily in the feces. In single-dose toxicity studies, the lethal SC dose in rats was more than 3700-fold the efficacious dose of moxidectin given as ProHeart 6 to dogs. In repeat-dose diet toxicity studies of durations up to 2 years in mice and rats, and 1 year in dogs, no target organs of toxicity were identified. There were no significant adverse histologic or biochemical effects on any organ system. There were no proliferative lesions identified in any tissue which may signal the

development of neoplasia, and no increase in tumors in mice or rats. The toxicity of moxidectin manifested itself at high doses in clinical signs such as lethargy, ataxia, tremors and mortality (rodents only) with concomitant decreases in food consumption and body weight. Such clinical signs of hypoexcitation are consistent with an exaggerated pharmacologic effect of moxidectin mediated via the GABA-A-receptor. Moxidectin was not genotoxic or carcinogenic and was without reproductive or developmental toxicity. The highest dosage evaluated in the 1-year dog study resulted in an estimated monthly exposure 234-fold the exposure observed after a single SC dose in dogs of ProHeart 6. Based on the toxicology studies of moxidectin where the dose, dosing duration, and resulting systemic exposure to moxidectin were significantly exaggerated, clinical SC administration of 0.17 mg/kg as ProHeart 6 to dogs is expected to be without significant adverse effects.

4.0 PROHEART 6 OVERVIEW

4.1 Clinical Trials with ProHeart 6 in Dogs

ProHeart 6 (moxidectin) is indicated for use in dogs ≥6 months of age for the prevention of heartworm disease caused by *Dirofilaria immitis* and for existing larval and adult hookworm infections (*Ancylostoma caninum* and *Uncinaria stenocephala*). The safety and efficacy of ProHeart 6 was demonstrated during a development program that was established in close collaboration with the FDA that included agreement on study requirements, protocol review, in-life inspections of selected development studies and a thorough review of data obtained during the program. Moxidectin is well established as an anti-parasitic product for cattle, sheep, horses, and dogs and is generally recognized as safe for these uses in all animals. The efficacy profile of ProHeart 6 was demonstrated in a series of dose-determination and dose-confirmation studies conducted in accordance with Good Clinical Practice guidelines. The safety of the product was evaluated through a series of safety studies in the target population as well as unique canine populations that would receive the product. These included reproducing males and females, dogs that had existing heartworm infections, and Collie dogs that had a demonstrated sensitivity to ivermectin. Safety and efficacy studies were conducted not only in laboratory Beagles but also in a variety of breeds and cross-breeds. In the sections that follow, dosages of ProHeart 6 expressed as mg/kg refer to mg of moxidectin per kg of animal bodyweight.

4.1.1 Heartworm

4.1.1.1 Dose Determination

Two (2) studies were conducted to determine the dosage of a single injection of ProHeart 6 required to effectively prevent *Dirofilaria immitis* infections for 6 months. Both studies had a similar experimental design. Study animals determined to be negative for *D. immitis* by antigen and a modified Knott's test were included in the studies. One study (0899-C-US-1-96, Georgia) utilized Beagle dogs (12 males and 20 females) and a second study (0899-C-US-2-96, Pennsylvania) utilized mongrel dogs (16 males and 16 females).[11,12] ProHeart 6 was administered as a single SC injection to dogs at a moxidectin dosage of 0.06, 0.17, or 0.50 mg/kg. Control animals received saline. At approximately 180 days (6 months) after treatment, all dogs were inoculated by intravenous (IV) injection with 50 *D. immitis* L_3 infective larvae. Infections were allowed to develop for 150 days, at which time each animal was euthanized, necropsied, and the heart and lungs removed for the recovery and quantification of heartworms. Immediately after treatment with ProHeart 6 and at various times throughout the studies, dogs were observed for adverse reaction to treatment. Additionally, injection sites were evaluated after treatment and throughout the study as well as histologically at necropsy.

In the Georgia study, an average of 25 worms were recovered in the saline treated controls. No worms were recovered from any of the dogs dosed with ProHeart 6, indicating that all dosages tested were 100% effective in preventing heartworm disease for 6 months.

In the Pennsylvania study, an average of 36 worms were recovered from the saline treated controls. No worms were recovered from any dog that received ProHeart 6 at 0.17 and 0.50 mg/kg. However, 14 adult *D. immitis* worms were found in 1 dog in the low-dose group (0.06 mg/kg). The results of this study indicated that a ProHeart 6 dosage of 0.17 mg/kg was appropriate to prevent canine heartworm disease for a period of 6 months.

During the dose determination studies, moxidectin concentrations were quantified in the serum of treated dogs. Following injection with ProHeart 6, peak moxidectin levels (approx. 5 ppb) were observed at 7 to 14 days post-treatment. At the end of the 6-month treatment period, residual moxidectin concentrations were negligible and generally below the limit of quantification of the assay methods. The drug did not accumulate with repeated doses. Serum

moxidectin levels evaluated after 4 treatments (6 months apart) confirmed that there was no accumulation of moxidectin in the serum of animals after successive doses (0899-C-US-9-98, Texas).[13]

4.1.1.2 Dose Confirmation

4.1.1.2.1 Adult Dogs

The efficacy of ProHeart 6 for the prevention of heartworm disease in dogs was confirmed in a series of studies conducted using the proposed commercial ProHeart 6 dosage of 0.17 mg/kg. Efficacy was evaluated at 6 months post-infection, 12 months post-infection and in dogs with existing heartworm infections to determine retroactive efficacy. The 6- and 12-month infection studies used similar experimental designs as the dose-determination studies. Animals were treated and challenged with L_3 infective larvae at either 6 or 12 months after treatment and necropsied for quantification of adult heartworms after the infections had matured. For activity against existing infections (retroactive efficacy), animals were challenged with L_3 infective larvae at various times prior to treatment with ProHeart 6 and necropsied after an appropriate period for the development of microfilariae to become adult heartworms.

ProHeart 6, administered to dogs at an SC dosage of 0.17 mg/kg, was 100% effective for prevention of heartworm disease for 6 or 12 months (0899-C-US-10-98, Texas).[14] When administered to dogs at an SC dosage of 0.17 or 0.27 mg/kg (0899-C-US-20-99, Pennsylvania), ProHeart 6 was 100% effective against development of canine heartworm disease for 12 months after treatment.[15]

The retroactive efficacy of ProHeart 6 with existing heartworm infections was evaluated at 4 and 6 months after challenge with L_3 infective larvae (0899-C-US-11-98, Georgia, Table 4.1.1.2.1-1).[16] At 0.17 mg/kg, ProHeart 6 was highly effective against 4-month-old *D. immitis* infections (85.9%). Efficacy was even higher (97.2%) against 4-month-old infections, when a second injection was given 6 months after the first injection; *D. immitis* was found in only 1 of 5 animals in this group. A single injection had low efficacy against 6-month-old infections (24.7%). Efficacy was not improved with 3 additional treatments 6 months apart. In a second retroactive study (0899-C-US-28-01, Georgia, Table 4.1.1.2.1-2), ProHeart 6 at 0.17 or 0.50 mg/kg was highly effective against 3-month-old infections (98.8 and 96.0% efficacy, respectively).[17]

Table 4.1.1.2.1-1. Retroactive Activity of ProHeart 6 at 0.17 mg/kg versus 4- and 6-Month Heartworm Infections

Treatment (Time Post-Infection in Months)		Geometric Mean Worm Counts (Percent Efficacy)		
Saline	**Moxidectin**	**Male**	**Female**	**Total**
4 and 6		14.07	17.31	31.66
	4	1.24* (91.2)	3.42* (80.3)	4.46* (85.9)
	6	12.09 (14.1)	11.61 (32.9)	23.85 (24.7)
4, 6, 10, 12, and 18		8.97	7.86	15.24
	4 and	0.32* (96.4)	0.25* (96.9)	0.43* (97.2)

*Statistically significantly different ($p < 0.05$) from control group based on analysis of geometric means.

Table 4.1.1.2.1-2. Retroactive Activity of ProHeart 6 versus 3-month Heartworm Infections

Treatment (mg/kg)	Geometric Mean Worm Counts (Percent Efficacy)		
	Male	**Female**	**Total**
Saline	16.0	22.0	38.3
0.17	0.3 (98.4)	0.3 (98.8)	0.4 (98.8)
0.50	0.6 (96.3)	1.1 (95.1)	1.5 (96.0)

Evaluation of efficacy was for male dogs only, where adequate infection was observed in control animals.

4.1.1.2.2 Puppies

When administered to 12-week-old small, medium and large breed puppies at 0.17 mg/kg (0899-C-US-30-02, Pennsylvania), ProHeart 6 reduced heartworm infection >99.8% compared with control puppies.[18] Treatment completely prevented heartworm infection in small and

medium breed puppies. One of six large breed puppies was infected with a single worm following challenge with 50 L_3 *D. immitis* infective larvae.

4.1.2 Hookworms

Efficacy was determined to be 100% for both larval and adult stages of *A. caninum* in three dose confirmation studies (0899-C-US-12-98, North Carolina; 0899-C-US-15-99, Georgia; 0899-C-US-16-99, Michigan).[19,20,21] Efficacy against both larval and adult *U. stenocephala* infections was 100% in study 0899-C-US-16-99.

Three (3) additional experimental infection studies were conducted to demonstrate the effectiveness of ProHeart 6 versus larval and adult stages of the hookworm *U. stenocephala*. Studies 0899-C-US-17-99 (Michigan), 0899-C-US-18-99 (New Jersey), and 0899-C-US-19-99 (Georgia) provided additional data that confirmed the efficacy of the product as >99.0%.[22,23,24]

Two studies were conducted to evaluate the persistent activity of ProHeart 12 against subsequent hookworm infections in dogs following treatment. In this program, animals were challenged with larvae of *U. stenocephala* and *A. caninum*. ProHeart 12 prevented infections of these 2 species of hookworm for a period of 4 months in a study in Georgia. In a study conducted in Michigan, ProHeart 12 prevented infection by *U. stenocephala* for a period of 8 months and *A. caninum* for 5 months.

4.1.3 Safety

4.1.3.1 Healthy Dogs

The clinical and possible pathological effects were evaluated when ProHeart 6 was administered to healthy dogs at either 1, 3 or 5 times the recommended dosage of 0.17 mg/kg in Study 0899-C-US-4-98 (Wisconsin).[25] Physical examinations were conducted prior to treatment and throughout the study. Blood and urine samples were collected for hematology, clinical chemistry, coagulation, and urinalysis. Dogs were also evaluated for clinical signs, food consumption, and body weight. At the end of the study, dogs were necropsied and evaluated for overt changes. Tissues from the control (saline treated) and 5-times dosage groups were examined microscopically. Dogs treated with ProHeart 6 did not demonstrate any signs or findings associated with the possible systemic toxicity of the drug. A single SC injection of

Proheart 6 equivalent to either 1, 3, or 5 times the commercial dosage caused swelling/slight edema at the site of injections starting within 8 hours of injection and lasting for up to 3 weeks. One dog, that received the 5-times dosage of ProHeart 6, displayed excessive salivation after treatment on Day 78. The only overt lesion observed was a 2.0 cm red focus at the SC injection site in 1 male at the 5-times dosage.

The safety of multiple injections of ProHeart 6 was studied in dogs given injections at 6 monthly intervals (0899-C-US-9-98, Texas) through 2 years.[13] No adverse reactions to treatment were observed following 5 injections. The injection sites were palpated externally, then the skin and underlying muscle tissue excised and examined. There were no gross findings at the injection sites. Microscopically, the injected areas generally had granulomatous panniculitis with microvacuolation (spheres) that was interpreted to be a reaction to the injected microspheres. Three (3) of the experimental dogs were maintained and received a total of 14 ProHeart 6 injections. At necropsy, there were no adverse reactions attributed to test article during this time, including no gross findings in the injection sites at necropsy.

The safety of ProHeart 6 was demonstrated in reproducing females (0899-C-US-3-98, Michigan) and males (0899-C-CN-1-98, Canada) at 3 times the recommended commercial dosage (0.5 mg/kg).[26,27] No adverse effects were observed in reproductive parameters of treated breeding females or the seminal quality of treated males.

When administered to healthy 10-week-old puppies at 3 or 5 times the recommended dosage rate of 0.17 mg/kg (0899-C-US-37-02, Michigan), ProHeart 6 caused no physical or neurological changes, and no changes in clinical chemistry or urinalysis parameters.[28]

4.1.3.2 Ivermectin-Sensitive Collie Dogs

Some genetic lines of Collie dogs are sensitive to the administration of ivermectin. A safety study (0899-C-US-13-98, Illinois) was conducted to determine the safety of a single dose of ProHeart 6 at 1, 3 and 5 times the proposed commercial SC dosage of 0.17 mg/kg.[29] Collie dogs shown to react to a 120 µg/kg bodyweight dosage of ivermectin (depression, ataxia, mydriasis, and excessive salivation) were enrolled in the study. Following treatment, dogs were observed intensively for the first 24 hours and twice daily through 21 days. There were no health conditions suggestive of toxicity in any of the dogs treated with ProHeart 6.

4.1.3.3 Heartworm-Positive Dogs

The safety of ProHeart 6 in heartworm-positive dogs was evaluated in 2 studies. The first study (0899-C-US-14-98, Alabama) tested a 3 times the proposed SC dosage in dogs with patent heartworm infections as measured by circulating microfilarial counts and heartworm antigen.[30] Clinical observations, physical exams, and microfilarial counts were used to evaluate the effects of treatment. The results demonstrated that a dosage of 0.51 mg/kg did not cause adverse reactions in dogs with patent heartworm infections. There were no post-treatment adverse health effects. Reduction in microfilariae compared with controls began as early as Day 7 post-treatment and peaked at 99.6% on Day 21 post-treatment. There was no significant ($p < 0.05$) adulticidal effect observed with ProHeart 6.

As part of the ProHeart 12 development program, the safety of this product for dogs was evaluated in dogs that had been surgically implanted with 20 adult heartworms via the jugular vein (0899-C-US-39-02, Georgia).[31] On Day 61 following implantation, dogs were treated with ProHeart 12 at 1.5 mg/kg (approximately 9 times the ProHeart 6 and 3 times the ProHeart 12 proposed commercial dosages). Animals were observed twice daily for signs of toxicity with physical and clinical examination. There were no treatment-related effects in any of the dogs. Microfilariae counts were reduced to almost 0 at 3 weeks after treatment, with no effect on the adult population demonstrating no adulticidal activity in heartworm-positive dogs.

4.1.4 Field Studies

The safety and efficacy of ProHeart 6 at 0.17 mg/kg was evaluated under field conditions in dogs dosed twice at 6-month intervals (0899-C-US-5-98, California; 0899-C-US-6-98, Texas; 0899-C-US-7-98, Wisconsin; 0899-C-US-8-98, Connecticut).[32,33,34,35] A total of 374 client-owned dogs representing 84 breeds (280 ProHeart 6 treated, 94 ProHeart oral tablet controls) completed the study. Dogs were ≥6 months of age and were of a variety of breeds, weights, physical condition, and were of both sexes (either intact or altered). Prior to enrollment, animals were tested for both heartworm antigen and microfilariae to ensure that dogs were negative for existing heartworm. None of the 374 dogs that completed this study tested positive for heartworms at either 3, 6, or 12 months after the initiation of ProHeart 6 treatment. The following potential adverse drug reactions were observed (number of cases): vomiting (3), diarrhea (2), weight loss (2), listlessness (1), seizures (1), injection site pruritus (3), and elevated

body temperature (1). Injection site evaluation revealed no abnormalities. This level of potential reactions for the 374 dogs that completed the study was extremely low and demonstrated the safety of the product under field conditions. Twelve (12) ProHeart 6 animals were euthanized or died during the 18-month study. It was determined following thorough review of each case that these deaths could not be attributed to treatment with ProHeart 6.

4.1.5 Exaggerated Moxidectin Overdose in Dogs

FDAH markets an oral formulation of moxidectin for use in horses to control internal parasites. This product is supplied as a syringe with a plunger calibrated by weight so that the correct dose can be given to horses varying in weight up to 1150 pounds.

While uncommon, dogs have been exposed to this product. Sometimes this exposure is intentional; owners believe it is cost effective and safe to administer the product to dogs. Occasionally dogs find discarded syringes and chew on them or they consume gel mixed with treats intended to entice a reluctant horse.

From 1997 to 2004, FDAH received approximately 250 reports. These cases were characterized by a wide range of neurological manifestations. Most (90%) of these dogs recovered to normal; the remaining 10% died. Thus, very high doses of moxidectin may lead to neurological manifestations of toxicity that are often reversible with no evidence of any long-term or non-neurological toxic effects

4.1.6 International Studies

ProHeart products have been registered in a number of international markets including Australia, Canada, the European Union (France, Greece, Italy, Portugal, Spain), Korea, and Japan. Length of activity claims and active ingredient concentration vary depending on the market. US data formed the basis of each registration with supporting local data, where required. These studies included dose-confirmation and field-efficacy testing.

Three (3) studies (0899-C-IT-01-99, 0899-C-IT-02-99, 0899-C-IT-03-99) were conducted in Italy to evaluate ProHeart 6 (trademark Guardian SR in Europe) at a dosage of 0.17 mg/kg for the prevention of heartworm disease caused by *Dirofilaria immitis* and *Dirofilaria repens*.[36,37,38] Two hundred and fifty-one (251) client-owned dogs of various breeds (41) completed this series

of field-efficacy and safety studies. ProHeart 6 given at 6 month intervals was 100% effective in protecting animals from heartworm infection in this heartworm endemic area (verified by untreated animals enrolled in the same area). No adverse reactions to treatment were reported.

In Australia, 3 studies (0899-C-AU-01-97 dose confirmation, 0899-C-AU-02-00 puppy safety, and 0899-C-AU-02-98 clinical field study) were conducted in support of a 12-month protection product administered at a dosage of 0.50 mg/kg.[39,40,41] ProHeart SR 12 was 100% effective in preventing heartworm disease in the laboratory dose-confirmation study. When administered at 3 times the recommended dose (9 times the ProHeart 6 dosage) in puppies 10 to 12 weeks of age, no clinically apparent adverse effects or adverse injection site reactions were observed. Breeds of dogs included Maltese cross, Lhasa Apso cross, Fox Terrier, Staffordshire Bull Terrier, Poodle, Border Collie, Labrador Retriever, German Shepherd and Rottweiler and represented small, medium, and large breeds. Two hundred and ten (210) animals representing 75 breeds completed the field clinical study. The study demonstrated the effectiveness of ProHeart SR 12 at 0.50 mg/kg (3 times the ProHeart 6 dosage) in protecting dogs from heartworm infection for 12 months. No adverse reactions or drug interactions were observed in treated dogs.

4.1.7 ProHeart 12

ProHeart 12, a moxidectin based product similar to ProHeart 6 is currently in development in the US. This product, administered at an SC dosage of 0.50 mg/kg will protect dogs from heartworm disease for a period of 12 months and is identical to the commercial product in Australia. A safety and field clinical program has been conducted. These studies demonstrate the same safety profile as ProHeart 6 in reproducing males and females, heartworm-positive dogs, ivermectin-sensitive Collies, and a variety of canine breeds during the field evaluation program.

4.1.8 Conclusion

The efficacy and safety of Proheart 6 has been thoroughly evaluated during the initial development program and in subsequent studies designed to expand label claims for the product. These programs were designed in close cooperation with the FDA and are comprehensive in scope. ProHeart 6 has been shown to be efficacious in the protection of dogs from heartworm disease. It is safe when administered to healthy dogs and to unique canine populations, such as heartworm-infected or ivermectin-sensitive dogs.

4.2 Postmarketing Surveillance of Adverse Events

FDAH designed a postmarketing surveillance system to detect a signal of potential drug effects and subsequently to estimate the incidence and causality of a potential drug effect. The data used for postmarketing surveillance generally consisted of adverse event reports (AERs).

To determine if AERs have clinical relevance, incidence is estimated and comparisons are made with a group representative of the general population that has not been treated with the drug. FDAH calculates reporting rates using the number of reports divided by an estimate of the doses sold to veterinarians for the same period. In contrast, regulatory authorities generally do not utilize estimates of the frequency of drug administration. Additionally veterinary epidemiologists lack an understanding of the rate of occurrence of even commonly observed medical problems. Thus, it is difficult in assessing AERs to determine whether a drug is directly related to the observed events. Even after determining that an adverse event may be likely related to drug administration, the clinical importance of the reaction is unknown without an understanding of its incidence. Frequent serious reactions may warrant withdrawal of a drug from the market, while rare reactions may require appropriate warnings to prescribers and clients.

Further confounding the interpretation of AERs is the potential for bias. There is a considerable opportunity for extraneous eventsto stimulate over-reporting of adverse observations in animals as possible drug associated adverse events. For ProHeart 6 in particular, there are additional biases that may have impacted reporting of adverse observations. Because ProHeart 6 is a new innovative product, veterinarians lack a frame of experience from which to judge whether or not adverse observations are likely to be associated with the drug. The long duration of action makes it appear plausible that adverse observations might be associated with drug administration that occurred several months earlier. Further, since ProHeart 6 is administered parenterally by veterinarians and the monthly oral medicines are administered by pet owners (often as a treat), there is a potential for greater apparent concern with ProHeart 6. Also, veterinarians received Dear Doctor letters discussing ProHeart 6 safety issues that may have stimulated AERs. Lastly, there were several widely-disseminated news reports and website postings critical of ProHeart 6 that may have stimulated AERs.

Thus, while postmarketing surveillance of AERs is a valuable tool in monitoring drug safety, the use of this system for regulatory decisions is complicated by the absence of a control group, the lack of an estimate of use, the lack of knowledge of the background rate of systemic diseases in the canine population, and the impact of reporting bias to stimulate over-reporting.[42,43,44]

4.2.1 Overview

In the subsequent sections, FDAH further evaluates AERs (1) relative to product usage, (2) relative to likelihood that they were associated temporally with ProHeart 6 administration, and (3) relative to independent expert assessment of the possible causality of the event as result of drug administration. On September 3, 2004, FDAH announced the voluntary recall of ProHeart 6 from the US market until resolution of FDA safety concerns. The primary concerns cited by FDA were the large number of AERs that were attributed to ProHeart 6 and that the numbers of these AERs were increasing in the months leading up to the recall. Additionally, the FDA expressed concerns regarding symptoms associated with several different body systems. These included neurologic, hematologic, hepatic, and cardiac systems, as well as a concern regarding neoplasia in association with ProHeart 6 administration.

FDA's website cites the submission of "more than 5500 AERs in compliance with federal regulations (21 CFR 514.80) that require sponsors to submit serious and unexpected AERs within 15 working days of first receiving the information." FDAH respectfully disagrees that all of these reports are medically serious or unexpected, or attributable to ProHeart 6. AERs defined as serious and unexpected are submitted to FDA within 15 working days. Those reports defined as not serious or not unexpected are normally submitted yearly in the annual Drug Experience Report. However, because of FDA's continuing concerns, FDAH began submitting all reports in a 15-day window in the third quarter of 2003, including those that were not serious and unexpected. Additionally, all events reported to FDAH were relayed to FDA in an unfiltered manner, regardless of causality assessment. Many AERs contain an assessment by the reporting veterinarians that their suspicion of product involvement in the event is low. This is particularly true for reports that were not deemed allergic events. FDAH has also assessed many of these events as having a low causal relationship. Thus, FDAH considers most of the AERs to be unrelated to ProHeart treatment.

Approximately 18 million doses of ProHeart 6 have been sold with more than 12 million doses administered. The calculated reporting rate is 2.5 reports per 10,000 doses, based on doses sold to veterinary clinics through August of 2004. This is the equivalent of one report (without regard to causality) per 4000 doses sold. It is recognized in a voluntary reporting system that not all potential adverse events associated with product use are reported. However, the trends over time need to be considered and indicate that the rate of AERs for ProHeart 6 was low and declined over time.

Another critical element is the background rate of disease in the canine population at large. The concerns regarding specific body systems are addressed in detail in section 4.2.2.2. Several independent experts were consulted and their conclusions, which are included for consideration, strongly support the safety of ProHeart 6 and indicate that most of the AERs were not causally-related to ProHeart 6 treatment.

One should consider the system used by FDA to assign causality to each AER. The six point causality assessment system considers previous experience with the drug, whether alternative etiologic candidates exist, timing of the event, overdose, and dechallenge and rechallenge. The system was primarily established for immediate release products. ProHeart 6 is a 6-month sustained-release product. Sustained-release products have not previously been evaluated using this system. Dechallenge (drug removal from the patient's body) is not possible for at least 6 months and FDA has acknowledged that the "challenge" and "dechallenge" components of their scoring system are not applicable in this instance. The modified scoring procedures to account for this fact may have created unintended bias among their reviewers.

4.2.2 Review of Adverse Event Reports

Upon receipt of an AER, the reporting party is asked a series of questions by FDAH professionals, including patient description and identification, previous history, date of product administration, its dose, body location of administration, time to observation of the adverse event, concurrent treatments employed, diagnostic testing, and event outcome. Regardless of causality assessment by FDAH, every AER involving an FDA regulated product received by FDAH is submitted to the FDA.

To assist in the analysis of AERs, FDAH developed an AER categorization system. Each AER is assigned into one or more of the following categories:

1. Injection site reports
2. Allergic reports (signs consistent with an allergic response in the species involved, occurring less than 48 hours after product administration)
3. Non-allergy systemic reports

To calculate reporting rates for evaluation of the incidence of events, the number of reports received is divided by the number of doses sold to veterinary clinics in the same period. The total number of vials of product sold is converted to an estimate of doses using an average number of doses per vial. Based on interviews with a large number of veterinarians, FDAH determined that the average number of doses per vial was 21. This dosage is equivalent to an 18 kg dog which is consistent with the average weight of the population at large.

There is a significant seasonality to the pattern of use of all heartworm preventative products. Figure 4.2.2-1 illustrates this seasonal pattern for doses of heartworm preventatives dispensed by veterinarians in the course of a typical year. This pattern is important to understand, when analyzing report numbers and reporting rates, as discussed below.

Figure 4.2.2-1. Seasonality of Dispensing Heartworm Medication 2002 (All Products)

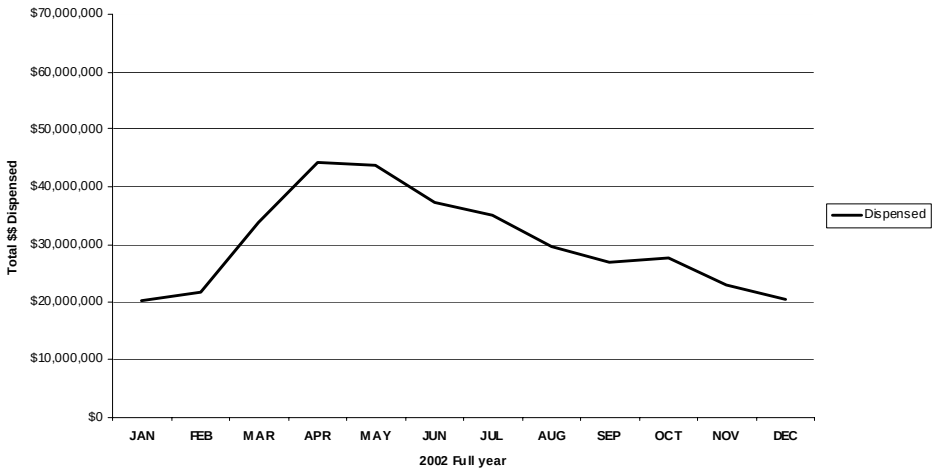


Figure 4.2.2-1 demonstrates the strong seasonal use patterns of heartworm preventative products in the US. The risk (and heightened awareness) of heartworm infection increases as mosquito populations either reappear after a typical northern winter, or crescendo after the more mild winters in southern areas of the US. Therefore, there is a large peak in use of all heartworm preventatives in April and May of the year.

Figure 4.2.2-2 demonstrates the sales patterns of ProHeart 6. Sales are increasing over the period. Sales peaks also occur in each year in essentially the same periods of time as the use patterns of heartworm products demonstrated in Figure 4.2.2.2-1.

Figure 4.2.2-2. Doses Sold by Quarter

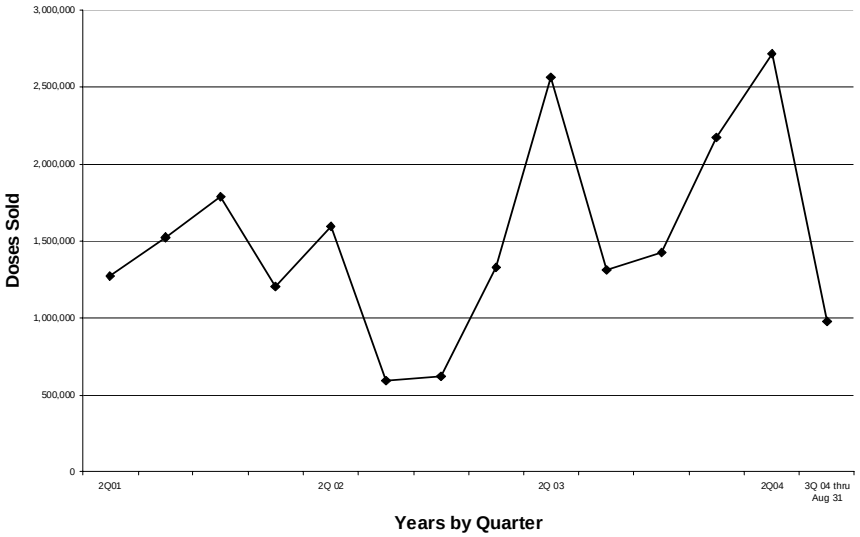


Figure 4.2.2-3 demonstrates the number of AERs received by FDAH associated with ProHeart 6. The number of AERs from launch through August of 2004 is generally decreasing. The peaks in the number of AERs in the second quarter of each marketing year correspond to the peaks in seasonal use and sales previously described. These peaks in AER numbers decrease in each marketing year despite large increases in sales of ProHeart6 shown in Figure 4.2.2-2. This seasonality in use, in conjunction with the increasing sales trends marketing dynamics of ProHeart 6, results in patterns of AER numbers that are difficult to interpret without proper perspective on the number of doses administered. Therefore reporting rates are calculated using report numbers found in Figure 4.2.2.-3 divided by sales data found in Figure 4.2.2.-3 to create Figure 4.2.2-4.

Figure 4.2.2-3. Number of Adverse Event Reports by Quarter

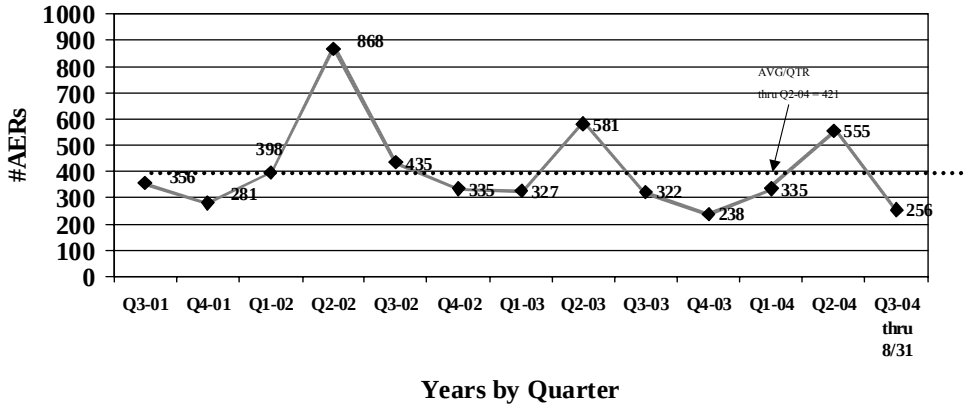
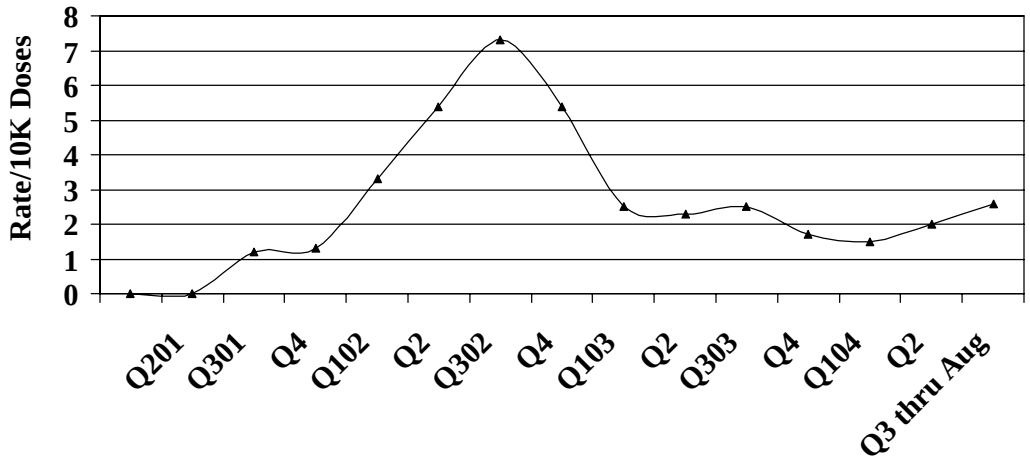


Figure 4.2.2-4. Reporting Rate by Quarter



The reporting rate shown in Figure 4.2.2-4 puts these trends into perspective. Marketing year two produced higher reporting rates, after which a more steady reporting rate was observed. Higher AERs were recorded in the 2nd quarter of 2002 (refer to Figure 4.2.2-3), but tapered off considerably following the August 2002 implementation of the manufacturing change of zero tolerance for residual solvents in the moxidectin used to manufacture ProHeart 6 (see Section 4.3). In the 2nd quarter of 2002, reporting rates peak not only due to an increased number of reports but also due to the return of large amounts of product in 2002, as a result of outdated product sold in 2001.

Evaluation by marketing year, which eliminates reporting rate fluctuations due to seasonality of use, reveals that the average reporting rate for AERs is less than 4 reports per 10,000 doses sold per year. By this same method of analysis, reports that include the death of the patient (regardless of causality) are fewer than 0.5 reports per 10,000 doses sold per year. Additionally, there is no peak in the number of death reports during the peaks in AERs seen in the 2nd quarter of each year. Further, the adverse event case fatality rate associated with ProHeart 6 reports is lower than many FDAH pharmaceuticals and similar to case fatality rates for the FDAH canine vaccine product lines including Duramune Max 5/4L. Thus the incidence of death does not appear to be causally related to ProHeart 6 usage.

Most AERs occurrred within 10 days of product administration. Overall, 72% of the AERs occurred within 48 hours of administration, (17%) were reported from 2 days to 10 days post-administration, and 10% occurred at 11 days or later. This information suggests that a significant percentage of reports occur in a short time frame, consistent with a possible allergic etiology from administration of ProHeart 6 alone or in combination with vaccines or other medication.

When the numbers of AERs were evaluated with consideration of the seasonality of use and the increases in the numbers of doses sold over time, the rate of reporting associated with ProHeart 6 was low and was decreasing with time. In addition, the seriousness of reports as estimated by case fatality rates was not increasing and was consistent with commonly used pharmaceutical and biological products. Thus, FDAH concludes that AERs were rare and the product should remain available as an alternative for prevention of canine heartworm disease.

4.2.2.1 Analysis by Report Category

4.2.2.1.1 Injection Site Reports

The reporting rate for injection site reports through 31 August 2004 was 0.2 reports per 10,000 doses sold. The majority of the events were self-limiting and consisted of swelling, pain and/or pruritus at the site of administration.

The occurrence of injection site reports after the use of ProHeart 6 has remained consistently low and the types of events reported were similar to other injectable products in the FDAH database. For comparison, 2 of the most widely used vaccines in the FDAH line have reporting rates of 0.1 reports per 10, 000 doses sold (Duramune Max 5/4L, a distemper/parvo/leptospirosis combination vaccine) and 0.5 reports per 10,000 doses sold (Rabvac 3, a rabies vaccine).

4.2.2.1.2 Allergy Event Reports

The allergy report category has trended down over time. The reporting rate through 31 August 2004 was 1.26 reports per 10,000 doses sold. The occurrence of allergy events was most prevalent in the most popular breeds, supporting the conclusion that there are no specific breed sensitivities. Thirty seven percent (37%) of the reports include concurrent vaccine

administration, which likely contributed to the reporting rate. For the vaccines previously cited, Duramune Max 5/4L has an allergy reporting rate of 0.4 reports per 10,000 doses sold and Rabvac 3 has an allergy reporting rate of 0.5 reports per 10,000 doses sold.

The occurrence of allergy events are described in the product label. The observations include facial swelling, angioedema, and urticaria, alone or in combination with gastrointestinal (GI) signs such as vomiting or diarrhea, anaphylaxis, or a low percentage of less common signs such as lethargy.

4.2.2.1.3 Non-Allergy Event Reports

Non-allergy events are those not considered to be an allergic response and not categorized as injection site. This non-allergy category includes systemic responses (regardless of the plausibility of product association) and may overlap with other categories. For example, an individual event report describing injection site swelling would be classified as an injection site report; however if fever and myalgia also occur, it would also be categorized as a non-allergy event. Similarly, there is an overlap in the symptoms designated as allergy and non-allergy. Acute GI symptoms occurring within 48 hours of product administration may be categorized as an allergic event, whereas the same symptoms occurring at 96 hours after product administration would typically be categorized as a non-allergy systemic event. Forty five percent (45%) of reports categorized as non-allergy occurred within 48 hours of product administration. In many cases, some of the clinical signs reported are consistent with allergy, while other signs vary enough to result in the event being placed in the non-allergy category. Therefore, FDAH concludes that a significant number of events placed in the non-allergy category are actually allergy mediated.

As observed in the allergy group, no specific breeds appear to be over-represented in the analysis when the population at large is considered. The overall reporting rate for the non-allergy category through 31 August 2004 was 1.19 reports per 10,000 doses. Concurrent vaccine use was reported in 42% of the non allergy reports. Duramune Max 5 /4L has 0.3 reports per 10,000 doses recorded in the non-allergy group and Rabvac 3 has 0.35 reports per 10,000 doses.

The signs reported in the non-allergy group cover a wide range, with a large number of the signs being reported in relatively few cases. The more commonly reported events include vomiting

and/or diarrhea, lethargy, seizures and ataxia. With the exception of ataxia, all of the above clinical signs are included in the product label.

4.2.2.2 Causality Analysis of Medical Events by Selected Body Systems

Events involving specific body systems were further analyzed to establish causality. These included events associated with neurologic reports, hematologic reports, hepatic reports, cardiac reports, and reports with a diagnosis of neoplasia.

Each ProHeart 6 case was individually reviewed to assess the likelihood that the observation was the result of administration of ProHeart 6. Medical association assessments were based on the approved International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) pharmacovigilance draft guidelines. These draft guidelines were developed with regulators from Europe, Japan, and the US to provide guidance on global pharmacovigilance harmonization. All events were assigned to the medical association "possible" category as a starting point. When the causality was considered "unlikely," sufficient information existed to establish that a reported event was not likely associated with product use because there were other more plausible explanations for the reported event. For the analysis by body system that are presented in the sections that follow only the medical association "possible" group was reviewed.

This medical association "possible" group was further reviewed to distinguish between events that could potentially be associated with a specific body system compared with those events for which it is probable that the specific body system was involved. Therefore, for each body system to be analyzed, 2 case definitions were developed: "potential" and "probable." The "potential" case definition is intended to include all clinical signs potentially reflective of an adverse effect on that body system. The "probable" case definition is more refined to include only those clinical signs that have a reasonable probability of being related to that body system, and the "probable" excludes all events classified as allergic. If any individual report included more than 1 of the body systems evaluated, it was included in multiple evaluations. For example, if the report involved a dog with anemia and elevated hepatic enzymes, it was reviewed under both the hematologic and hepatic body systems.

4.2.2.2.1 Neurologic

The analysis of neurologic events is hown in Table 4.2.2.2.1-1. The rate of reporting is low at 0.12 reports per 10,000 doses. The difference between the number of potential and probable neurologic events is relatively large because events classified as allergic and events with non-specific neurologic signs are included in the number of potential events but not the number of probable events. Non-specific neurologic signs are clinical signs (eg, muscle tremors) that are seen without other clinical signs and, therefore, are not likely to include a primary neurologic disorder.

For neurologic events, seizure and ataxia are the primary clinical signs while other individual clinical signs each account for less than 10% of the total in the neurologic group. As is true with each of the systems, causality assessments are confounded by concurrent vaccine use in 47% of the AERs. Additionally, 54% of the reported events occurred within 48 hours or less, suggesting that many may be allergic events.

Table 4.2.2.2.1-1. Neurologic Adverse Events – Number of Reports

Year	Potential	Probable	Rate (Probable) per 10K Doses Sold
2001	98	23	0.05
2002	342	85	0.21
2003	235	69	0.10
2004-Aug	211	68	0.12
Total	886	245	0.12

The "probable" neurologic reports were reviewed by Dr. Alexander deLahunta D.V.M., PhD., diplomate of the American College of Veterinary Internal Medicine, specialty neurology and honorary member of the American College of Veterinary Pathologists (Appendix 6.2). FDAH randomly selected about 75% of the neurologic AERs for his review. Dr. deLahunta noted that seizures were the most common clinical sign reported in the cases, which is consistent with the general observations in the practice of clinical neurology. He also commented that the most common cause of seizures in dogs is idiopathic epilepsy. Dr. deLahunta concludes his assessment with the following statement: "The extensive toxicologic studies performed by

FDAH clearly support the safety of ProHeart 6 at the dose injected in these dogs. There is no rational scientific reason to believe that this product played any role in the post-injection clinical signs reported in these case summaries."

4.2.2.2.2 Hematologic Events

The analysis of hematologic events is shown in Table 4.2.2.2.2-1. The rate of reporting is low at 0.09 reports per 10,000 doses. As is true with each of the systems, causality assessments are confounded by concurrent vaccination in 49% of the AERs.

Table 4.2.2.2.2-1. Hematologic Adverse Events – Number of Reports

Year	Potential	Probable	Rate (Probable) per 10K Doses Sold
2001	75	13	0.03
2002	212	63	0.16
2003	172	48	0.07
2004-Aug	139	60	0.10
Total	598	184	0.09

FDAH consulted with Dr. Alan Rebar, a diplomate of the American College of Veterinary Pathologists with a specialty in clinical pathology, to review all potential hematologic events (Appendix 6.3). Dr. Rebar was provided with clinical case summaries and all available clinical laboratory data for all the clinical cases identified by FDAH criteria as probable hematologic or hepatic adverse events to ProHeart 6. (The review of the hepatic events follows in Section 4.2.2.2.3.)

In contrast to FDAH's assessment (Table 4.2.2.2.2-1), Dr. Rebar determined that 151 of the AERs he reviewed should be categorized as probable hematologic AERs. Particular emphasis was placed on potential toxic-induced hematologic events: immune mediated hemolysis (IMHA), immune mediated thrombocytopenia (ITP), Heinz body hemolysis, and bone marrow hypoplasia /aplasia (IMMD). Of these, only IMHA, ITP and/or IMMD were identified among the 151 hematologic reports.

Dr. Rebar also evaluated the 151 probable hematologic events to determine which may have been immune mediated. Using a conservative case definition he determined that 79 could be immune mediated. In many of the 79 cases, however, not enough data were provided to establish a definitive diagnosis of IMHA, ITP or IMMD. For example, in many cases, blood films were not evaluated for the presence of spherocytes, direct antiglobulin tests (DAT, Coombs' tests) were not run, and blood films were not scanned to confirm an instrument reported thrombocytopenia.

Of the 79 cases determined to be potentially immune mediated, 76 cases were determined to be IMHA/ITP. Of these, 53 cases occurred after the first injection of ProHeart 6, and 26 occurred after 2 or more injections. Of the 53 cases occurring following a single ProHeart 6 injection, only 23 (those occurring 8 to 30 days post-injection) were considered possibly related to ProHeart 6. Fourteen (14) occurred too soon following ProHeart 6 injection (0 to 7 days) to have allowed sufficient antibody to form against ProHeart 6 to precipitate an immune-mediated hematologic event. Thirteen (13) occurred after 30 days or more, too long an interval to suggest a likely role for ProHeart 6 injection.

Of the remaining 23 cases, it is not possible to rule out a role for ProHeart 6, although IMHA and ITP are relatively common disorders in all breeds of dogs.

Of the 26 cases occurring following multiple injections of ProHeart 6, twenty two (22) (those occurring between 0 and 30 days post ProHeart 6 injection) were considered possibly related to ProHeart 6. The 10 cases occurring within 7 days post-injection are relatively unlikely to be related to ProHeart 6 injection but are included because of potential anamnestic immune response to multiple injections.

Three (3) cases of apparent IMMD were identified. The cause of the IMMD in each case could not be determined from the diagnostic data available. Two (2) cases occurred after the first dose of ProHeart 6; the first occurred at 7 days post administration and the second occurred at more than 30 days post administration. The third case occurred more than 30 days after the dog had received its fourth dose of ProHeart 6. The timing of all 3 events indicates that they were unlikely to be related to ProHeart 6 administration.

In summary, 45 cases of hematologic AERs may have been induced by ProHeart 6. However, since more than 18 million doses of ProHeart 6 have been sold it is possible that these findings represent the normal baseline incidence in canines.

4.2.2.2.3 Hepatic Body System

The analysis of hepatic events is shown in Table 4.2.2.2.3-1. The rate of reporting is low at 0.07 reports per 10,000 does sold. As is true with each body system, causality assessments are confounded by concurrent vaccine use in 38% of the AERs.

Table 4.2.2.2.3-1. Hepatic Adverse Events – Numbers of Reports

Year	Potential	Probable	Rate (Probable) per 10K Doses Sold
2001	36	12	0.03
2002	125	48	0.12
2003	109	49	0.07
2004-Aug	118	47	0.08
Total	388	156	0.07

One hundred (100) probable hepatic AERs were reviewed by Dr. Rebar. This number of 100 probable hepatic reports is different from the total probable hepatic reports in Table 4.2.2.2.3.-1 because Dr. Rebar removed 56 reports that he determined were not probable hepatic events. Forty-three (43) cases of possible primary liver disease were identified among the 100 AERs evaluated. Fifty-seven (57) cases were probably not primary hepatic events, and the mild non-specific elevations in hepatic enzyme(s) were thought to be due to anorexia and/or stress.

Of the remaining 43 cases, 42 occurred between 0 and 3 days post- injection; again, however, a causal relationship with ProHeart 6 cannot be ruled out. However, since more than 18 million doses of ProHeart 6 have been sold it is possible that these findings represent the normal baseline in the canine population.

Pathology reports from all the probable hepatic AERs that contain any pathology reports (15 cases) were reviewed by Dr. Keith Harris D.V.M., Assistant Vice President Pathology and Bioresources, Wyeth Research and diplomate of the American College Veterinary Pathologists. There was no pattern of liver pathology indicative of a common toxicological agent in all or a subset of the 15 cases (other than corticosteroids in two cases). Only one case out of the 15 exhibited histomorphologic changes consistent with a direct acting hepatotoxin that could be temporally related to ProHeart 6 administration. Pathology reports from 15 cases with liver disease were reviewed. There was no obvious pattern of liver pathology that would indicate toxicity common to all or a subset of the 15 cases (other than corticosteroids in two cases). Only one case out of the 15 exhibited histomorphologic changes consistent with a direct acting hepatotoxin that could be temporally related to ProHeart 6 administration. The acute hepatocellular necrosis described in this particular case is a non-specific finding and could have been caused by a number of different toxicants.

4.2.2.2.4 Cardiac Events

The analysis of cardiac events is seen in Table 4.2.2.2.4-1. The reporting rate is low at 0.02 reports per 10,000 doses.

Table 4.2.2.2.4-1. Cardiac Adverse Events – Numbers of Reports

Year	Potential	Probable	Rate (Probable) per 10K Doses Sold
2001	100	7	0.02
2002	254	15	0.04
2003	186	10	0.02
2004-Aug	153	15	0.02
Total	693	47	0.02

Concurrent vaccination confounds the assessment of the cardiac events. Forty-nine percent (49%) of the cases classified as cardiac events included concurrent vaccination. Sixty percent (60%) of the reports occurred within 48 hours of ProHeart 6 administration. The timing of these

reports is not indicative of a direct cardiac effect. Many events appear to overlap as non-typical allergic events, as observed for the other body systems, so that an indirect effect associated with an allergic event cannot be ruled out.

Dr. Keith Harris DVM, Assistant Vice President Pathology and Bioresources Wyeth Research, and diplomate of the American College of Veterinary Pathologists, reviewed the histopathological slides from 2 cases of cardiac necrosis thought to be associated with ProHeart 6 administration. Histopathologic evaluation of tissue specimens from the 2 dogs identified in an FDA presentation revealed cardiac pathology secondary to uremia in a Boxer dog and chronic heart disease that clearly predated ProHeart 6 administration in a Labrador Reteiever. The two IDEXX pathologists who had originally indicated that these cases might be associated with ProHeart 6 subsequently concurred with this interpretation.

Based on these data, including timing of the events and histopathological assessments, FDAH concludes that there is no causal relationship between ProHeart 6 and these cardiac events.

4.2.2.2.5 Neoplasia Events

The analysis of neoplasia cases is shown in Table 4.2.2.2.5-1. The rate of reporting is low at 0.06 reports per 10,000 doses.

Table 4.2.2.2.5-1. Neoplasia Adverse Events – Numbers of Reports

Year	Potential	Rate per 10K Doses Sold
2001	7	0.01
2002	38	0.09
2003	41	0.06
2004-Aug	36	0.06
Total	130	0.06

Dr Philip Bergman, D.V.M, MS, PhD, diplomate American College of Veterinary Internal Medicine, Oncology (Appendix 6.4) reviewed all 130 cases involving a diagnosis of neoplasia, regardless of medical association. A summary of Dr. Bergman's comments follows.

The vast majority of ProHeart 6-related cancers occurred within 21 days or less of ProHeart 6 administration. Veterinary oncologists agree that at least 6 to 8 weeks are required to develop a tumor after exposure to a carcinogen. Therefore, cases that were diagnosed with cancer within a 3 to 4 week period after ProHeart 6 administration likely had a tumor present before ProHeart 6 administration. There was not a relationship between additional doses of ProHeart 6 administered and increasing reports of neoplasia. This lack of a dose-response relationship with repeated ProHeart 6 administration further supports the conclusion that ProHeart 6 is not responsible for canine tumor induction. This is further supported by the moxidectin nonclinical toxicology studies showing no evidence of carcinogenicity (see Section 3.3.3 and Appendix 6.1).

Dr. Bergman's review of the 130 cases revealed that 100 cases were clearly not associated with ProHeart 6 administration. Twenty eight (28) cases did not allow the immediate exclusion of an association to ProHeart 6 administration. However the association was deemed unlikely due to 1 or more of the following factors: 1) a time period from product administration to observation of the neoplasia was too short to be considered a causal relationship; 2) most of the reports revealed no association between the site of administration and the location of the neoplasia; 3) there was a broad cross section of neoplasia types, rather than a predilection for a specific tumor type, as would be expected in a product-specific effect; and 4), the types were representative of those seen in the general dog population. There were 2 cases in which a relationship to ProHeart 6 administration could not be ruled out.

4.2.3 Conclusions

Based on the in-depth analysis described in this summary section, it is the conclusion of FDAH that ProHeart 6 is a safe and effective product for the prevention of canine heartworm disease. The overall reporting rate for AERs was low and generally trended down over time to the time of the recall. At that time, the frequency and severity of AERs was not increasing. Analysis of specific body and organ systems support the conclusion that the AER clinical finding were generally not causally related to ProHeart 6 administration and appeared to occur at a rate consistent with naturally occurring disease in the canine population.

4.3 Manufacturing Change

ProHeart 6 was launched in the US in June 2001. Shortly after launch, FDAH received a number of reports of allergic-type reactions after administration. The reactions reported ranged from mild and self-limiting (eg, urticaria, itching at injection site, an episode of vomiting or diarrhea) to severe anaphylactoid reactions.

A working group of FDAH and Wyeth immunology experts was convened to investigate the possible causes, and outside experts were retained to assist. There were 2 aspects of the investigations: (1) field reports and their follow-up, and (2) product analysis for any abnormalities, contaminants, and/or reactive components.

A "cluster effect" was observed where some practices reported several adverse events, while others in close proximity with usage patterns of ProHeart 6 did not report reactions. This was observed in both the US and Australia. Visits were made to practices to evaluate product storage, handling, and administration, and veterinarians and dog owners were interviewed to try to identify any predisposing factors. No age or breed predispositions were found, nor any interactions with concurrent treatment with other veterinary therapeutics or biologicals.

Extensive investigations into reactor dogs (dogs which showed allergic reactions) found no evidence that the allergies were IgE (immunoglobulin E) or IgG (immunoglobulin G) mediated (L. Gershwin, Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, and R. Schultz, Professor and Chair, School of Veterinary Medicine, Department of Pathological Sciences, University of Wisconsin, Madison [see Appendix 6.5]). The reactions appeared to be idiosyncratic and mediated either by histamine or complement.[45,46,47] Attempts to induce reactions in dogs using passive cutaneous allergy testing after sensitization with serum from reactor dogs were unsuccessful.[48,49]

Extensive testing of all raw materials and formulated products used in manufacture of ProHeart 6 showed that all were within quality specifications. Tests for 16 trace metals did not reveal any contamination. Quality Assurance audits were conducted at the manufacturing sites of glyceryl tristearate (Germany) and hydroxypropyl methylcellulose (US) to evaluate the potential for cross contamination during or after manufacturing. No potential problems were found. Cleanout

validation was repeated at the formulation plant to confirm that no carryover from other manufacturing processes could occur.[50,51,52]

Both in the US and Australia, differences were found in reaction rates between batches, so investigations focused on trying to identify any batch differences. Thin layer chromatography (TLC) analyses detected no unknown components either in moxidectin technical material or in finished product. However, evaluation of minor component profiles between batches revealed a trend to lower reactions for lots with no detectable residual solvents. Coincident with this investigation, FDAH was optimizing the manufacturing process and initiated a manufacturing change to produce moxidectin with no detectable solvents.

Although additional studies were done to better understand the cause of the allergic-type reactions, no conclusive findings were generated. Nevertheless, there has been a decline in the adverse event reporting rate from all markets since the manufacturing change was implemented.

4.4 Safety Profile in Dogs

In August of 2004, FDAH requested a review of the safety of ProHeart 6 use in general veterinary practice and a comparison of its safety profile with that of commonly used oral monthly heartworm drugs by Dr. Larry Glickman VMD, DrPh, FACE, Professor of Epidemiology and Public Health and Head, Section of Clinical Epidemiology, School of Veterinary Medicine, Purdue University (Appendix 6.6). This section summarizes the experience with ProHeart 6 by Banfield the Pet Hospital™ veterinarians nationwide who had administered 735,654 doses of ProHeart 6 to dogs from January 1, 2002 through August 31, 2004.

4.4.1 Methods

The source of data for this analysis was the medical records of Banfield the Pet Hospital™. Banfield was founded in 1955 in Oregon to deliver primary health care to companion animals, and by 2005 will operate a national network of 440 full-service veterinary hospitals in 42 states. Banfield practices employ more than 900 full and part-time veterinarians, have over 1.4 million active patients, and conduct approximately 50,000 patient visits per week. The Banfield database is paperless and contains over 8 million patient records in electronic format. A quality assurance team consisting of veterinarians and veterinary technicians regularly monitors the safety profile of all medications, vaccines, and procedures used by Banfield veterinarians and tracks the incidence of diseases that are preventable by these vaccines or drugs.

All encounters (office visits) were characterized as being associated with ProHeart 6, 2 oral monthly heartworm preventative drugs (Heartworm Preventative 1 or Heartworm Preventative 2), vaccine, or none of these exposures. Adverse events (AEs) of interest included liver disease, neurological disease, ocular disease, immune-mediated disease, allergic reaction, death, cancer (mast cell, lymphosarcoma, and histiocytoma), cardiovascular disease, anaphylaxis, or inflammatory bowel disease. The specific diseases or laboratory findings comprising each of these AEs based on Banfield computer codes are shown in Table 4.4.1-1. Each encounter was then evaluated for potential AEs over the following 30 days. Of these encounters, 275,189 that occurred from August 1 to August 31, 2004, were excluded from analysis because they lacked a full 30-day followup interval. This followup period was

terminated early if a dog had another exposure to ProHeart 6, either of the 2 monthly heartworm preventatives, a vaccine, or died. Except in the case of death, a new followup period was initiated after this new exposure. The incidence of AERs, expressed as either the number of AEs per 10,000 encounters or the number of AE per 10,000 days at risk following an exposure, were calculated for the following exposure groups: no exposure, vaccine alone, ProHeart 6 with or without vaccine, and Heartworm Preventative 1 or Heartworm Preventative 2 with or without vaccine. Formal testing to identify statistically significant differences in the AE rates between exposure groups was generally not done for the univariate analyses due to the very large sample size within each exposure group. That is, the power to detect statistically significant differences was so high for common events that even very small differences in event rates were likely to be statistically different at $p < 0.001$. However, the same was not necessarily true for less common events. For this reason, the focus was placed on the clinical relevance of differences in the AE rates and the 95% confidence intervals of the odds ratios in multivariate analysis.

Table 4.4.1-1. Adverse Events

Disease Category	Adverse Event	Identification Criteria
Liver Disease	liver any dx	dx = hepatopathy, hepatitis, hepatic enceph, hepatic acute, hepatic dis, hepatic conserv, hepatic extensv
	liver alp	lab = alp >= 393
	liver alt	lab = alt >= 236
	liver gam	lab = gamma gt >= 24
	liver bil	lab = tot bilirubin > 1.0
	liver lab	any liver adverse lab code
	liver any dx + any lab	any liver adverse dx + any liver adverse lab code
	liver any	any liver adverse dx or any liver adverse lab code
Neurological Disease	neuro	dx = enceph mening, epilepsy, behavioral uk, shock-cardio, seizures-acq lab = paresis, paralysis, ataxia
Ocular Disease	ocular	dx = optic neuritis, retinal-degen-s, anisocoria lab = vis acuity, vis deficit-lft, visual deficit-rgt
Immune Mediated Disease	Thrombocytopenia	dx = thrombocytopenia, thrombo im
	immun_med1	dx = (immune med dis or AHA) and lab = abnormal reticulocyte count
	immun_med2	dx = (immune med dis or AHA) and not lab = abnormal reticulocyte count
	immune any	any immune mediated disease adverse event
Allergic Reaction	allergic reaction	dx = allergic reaction, drug reaction, drug induc dis, allergic rct acut, vaccine reaction, urticaria, drug eruption
Death	death	dx = dead on arrival, sudden death death date within 30 days based on reported death in demographics records
Anaphylaxis	anaphylaxis	dx = anaphylaxis
Cardiac	cardiac murmur	dx = murmur
	cardiac arrhythmia	dx = cardiac arrest, atrial fibrillation, atrial premature contractions, atrial tachycardia, bundle branch block, heart block 1st deg, heart block 2nd deg, heart block 3rd deg, cardiac arrhythmia, ventricular premature contractions, ventricular tachycardia
	cardiomyopathy	dx = cardiomyopathy, canine dilated, cardiomyopathy; canine hypertrophic, cardiomyopathy; boxer, cardiomyopathy; canine familial, cardiomyopathy; dilated, cardiomyopathy; hypertrophic
	cardiac any	any cardiac adverse dx
Cancer	mast cell tumor	dx = mast cell tumor
	lymphosarcoma	dx = lymphosarcoma
	histiocytoma	dx = histiocytoma
	cancer any	any cancer adverse dx
Inflammatory Bowel Disease	ibd	dx = inflammatory bowel disease

For the purposes of this study, it was assumed that oral monthly heartworm preventatives had been administered by owners on the same day of the encounter in which they had been dispensed. In contrast, ProHeart 6 and vaccines were assumed to have been administered during the same office encounter indicated in the medical record. Also, a search for potential AEs was limited to the 30 days immediately following an encounter of interest. The potential consequences of these assumptions would be to underestimate the incidence of AEs associated with oral monthly heartworm drugs, compared with those associated with ProHeart 6 or vaccines, because it was not certain if, or when, the monthly heartworm preventative dispensed for a dog was actually given to the dog by its owner. That is, some of the oral heartworm preventatives dispensed by Banfield veterinarians were probably never given to the dogs, yet AEs were calculated as if these drugs had been administered.

4.4.2 Results

From January 1, 2002 to August 31, 2004, there were 6,800,061 dog visits or encounters at 403 Banfield hospitals. The following number of encounters was associated with the administration of: ProHeart 6 (735,654), Heartworm Preventative 1 (411,082), Heartworm Preventative 2 (18,405), or vaccine (2,230,202), (Table 4.4.2-1). In addition, there were 5,634,016 encounters during which no heartworm preventative was administered or dispensed. Heartworm preventatives were often administered or dispensed at the same time as a vaccine. The proportion of encounters associated with vaccination was 62.9% for Proheart 6, 59.9% for Heartworm Preventative 1, and 65.1% for Heartworm Preventative 2. In contrast, vaccines were only administered during 26.4% of the encounters for which no heartworm preventative was given. That is, these dogs may have presented with signs of disease for which diagnostic tests were performed and other drugs administered. The number of doses of ProHeart 6 administered monthly by Banfield veterinarians increased over time since January 2002 and a higher number of doses was administered each year during the peak time of mosquito activity, namely from March through September (Figure 4.4.2-1).

Table 4.4.2-1. Rate per 10,000 of Any Adverse Event by Treatment Category Total Number of Encounters = 6,800,061

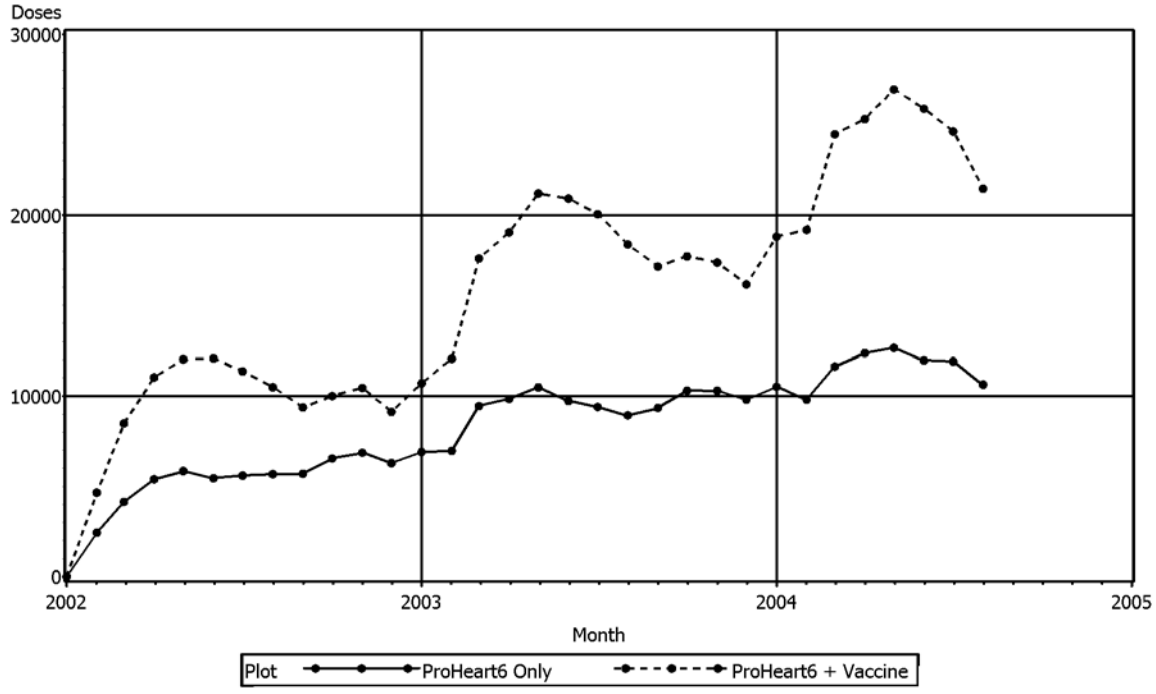
	ProHeart6			HW Prev 1			HW Prev 2			No HW Treatment		
Vaccine	N	N_A	Rate	N	N_A	Rate	N	N_A	Rate	N	N_A	Rate
Yes	483,064	6,292	130.3	246,131	2,804	113.9	11,975	120	100.2	1,489,032	17,406	116.9
No	252,590	2,253	89.2	164,951	1,469	89.1	6,430	45	70.0	4,144,984	120,529	290.8
Total	735,654	8,545	116.1	411,082	4,273	103.9	18,405	165	89.4	5,634,016	137,935	244.8

N = Total Number of Encounters

N_A= Total Number of Adverse Events

Rate = Number Adverse Events Per 10,000 Encounters

Figure 4.4.2-1. Doses Dispensed by Month; ProHeart 6 Only or With Vaccine



4.4.2.1 Univariate Analyses

ProHeart 6 was associated with a higher rate of liver-related AE compared with either of the 2 monthly heartworm preventatives, regardless of whether a vaccine was administered or not, while Heartworm Preventative 1 was associated with the highest death rate (Table 4.4.2.1-1). In general, vaccine administration was associated with an increased rate of liver disease for dogs receiving ProHeart 6, but not for dogs on monthly heartworm preventative. The rates of all other diseases or conditions except for cancer were similar among all of the heartworm preventatives, whether given orally or parenterally.

Table 4.4.2.1-1. Adverse Event Rate per 10,000 by Treatment Group

Treatment Category					Potentially Associated Adverse Event Type									
					Liver Disease		Neurological Disease		Ocular Disease		Immune Mediated Disease		Allergic Reaction	
ProHeart6	HW Prev 1	HW Prev 2	Any Vaccine	N	N	Rate	N	Rate	N	Rate	N	Rate	N	Rate
				4,144,984	25,531	61.6	3,157	7.6	134	0.3	2,948	7.1	6,832	16.5
			Y	1,489,032	5,207	35.0	522	3.5	27	0.2	265	1.8	6,598	44.3
		Y		6,430	14	21.8	4	6.2	0	0.0	1	1.6	12	18.7
		Y	Y	11,975	24	20.0	2	1.7	0	0.0	2	1.7	62	51.8
	Y			164,951	523	31.7	68	4.1	1	0.1	40	2.4	236	14.3
	Y		Y	246,131	671	27.3	94	3.8	2	0.1	22	0.9	1,336	54.3
Y				252,590	880	34.8	123	4.9	4	0.2	59	2.3	465	18.4
Y			Y	483,064	2,011	41.6	178	3.7	7	0.1	98	2.0	2,581	53.4
Any				6,800,061	34,866	51.3	4,149	6.1	175	0.3	3,435	5.1	18,123	26.7

Treatment Category					Potentially Associated Adverse Event Type									
					Death		Cancer		Cardio Disease		Anaphylaxis		IBS	
ProHeart6	HW Prev 1	HW Prev 2	Any Vaccine	N	N	Rate	N	Rate	N	Rate	N	Rate	N	Rate
				4,144,984	74,470	179.7	3,724	9.0	8,089	19.5	139	0.3	767	1.9
			Y	1,489,032	2,690	18.1	562	3.8	1,804	12.1	108	0.7	62	0.4
		Y		6,430	11	17.1	0	0.0	4	6.2	0	0.0	1	1.6
		Y	Y	11,975	17	14.2	2	1.7	10	8.4	2	1.7	0	0.0
	Y			164,951	363	22.0	67	4.1	219	13.3	4	0.2	17	1.0
	Y		Y	246,131	354	14.4	75	3.0	261	10.6	18	0.7	17	0.7
Y				252,590	392	15.5	155	6.1	250	9.9	13	0.5	19	0.8
Y			Y	483,064	709	14.7	205	4.2	635	13.1	34	0.7	26	0.5
Any				6,800,061	79,006	116.2	4,790	7.0	11,274	16.6	318	0.5	909	1.3

The rate of potential liver-related AEs was examined further on the basis of a clinical diagnosis only, an increase in the serum concentration of a liver-associated enzyme or bilirubin, or any combination of a clinical diagnosis plus an abnormal laboratory finding (Table 4.4.2.1-2). ProHeart 6 when administered either with or without a vaccine, was associated with a higher rate of any liver-related clinical diagnosis, enzyme ALT, bilirubin, or a combination of a clinical diagnosis plus any abnormal laboratory test result (Table 4.4.2.1-3). However, in an analysis based on the rate of potential AEs per 10,000 days at risk, the rate of liver disease was comparable between ProHeart 6 and the 2 monthly heartworm preventatives, while the rate was highest for dogs that received no heartworm drug. It should be noted that the mean days at risk per encounter for ProHeart 6 was 29.2 compared with 27.2 for Heartworm Preventative 1. This could lead to a slight underestimation of the AE rate associated with Heartworm Preventative 1 compared with ProHeart 6. The liver-related AEs appeared to occur with similar frequency during days 0 to 2, 3 to14, and 15 to 30, following the exposures of interest.

Table 4.4.2.1-2. Liver Adverse Events Definitions Based on Lab Measure or Lab Abnormal Flag

Measure	Criterion	Times Normal	Lab Measure N	Lab Abnormal Flag N
ALP	>= 393	3X	22,913	37,869
ALT	>= 236	2X	12,201	32,406
Tot Bilirubin	>= 1.0	3X	24,823	27,049
Gamma GT	>= 24	2X	485	14,092
Total			**60,422**	**111,416**

Table 4.4.2.1-3. Potential Liver-related Adverse Events per 10,000 by Treatment Group

Treatment Category					Potentially Associated Adverse Event Type															
	HW	HW	Any		Any DX		ALT		ALP		GAM		BIL		Any LAB		DX + LAB		DX or LAB	
PH6	Prev 1	Prev 2	Vaccine	N	N	Rate	N	Rate	N	Rate	N	Rate	N	Rate	N	Rate	N	Rate	N	Rate
				4,144,984	3,776	9.1	6,616	16.0	12,422	30.0	391	0.9	8,754	21.1	23,536	56.8	1,452	3.5	25,531	61.6
			Y	1,489,032	460	3.1	1,006	6.8	1,703	11.4	20	0.1	2,688	18.1	4,997	33.6	218	1.5	5,207	35.0
		Y		6,430	0	0.0	4	6.2	3	4.7	0	0.0	9	14.0	14	21.8	0	0.0	14	21.8
		Y	Y	11,975	0	0.0	8	6.7	8	6.7	0	0.0	13	10.9	24	20.0	0	0.0	24	20.0
	Y			164,951	52	3.2	118	7.2	201	12.2	8	0.5	237	14.4	498	30.2	20	1.2	523	31.7
	Y		Y	246,131	45	1.8	94	3.8	173	7.0	3	0.1	416	16.9	645	26.2	15	0.6	671	27.3
Y				252,590	100	4.0	215	8.5	326	12.9	7	0.3	400	15.8	836	33.1	43	1.7	880	34.8
Y			Y	483,064	206	4.3	389	8.1	696	14.4	8	0.2	984	20.4	1912	39.6	95	2.0	2,011	41.6
Any				6,800,061	4,641	6.8	8,450	12.4	15,536	22.8	437	0.6	13,502	19.9	32,467	47.7	1,845	2.7	34,866	51.3

Vaccine administration was associated with a markedly increased rate of allergic reactions for dogs on ProHeart 6 as well as for dogs on monthly heartworm preventative (Table 4.4.2.1-1). The rate of allergic reactions per 10,000 days at risk was similarly increased for vaccinated dogs compared with dogs receiving any one of the heartworm preventative drugs or those receiving no heartworm drug. Unlike liver-related AEs, allergic reactions occurred more commonly during days 0 to 2 following an encounter of interest.

There was no apparent association of immune-mediated or cardiovascular events with any of the heartworm preventatives or with vaccines, and the rate of these AEs was relatively low (Table 4.4.2.1-1).

The rate of mast-cell tumor, lymphosarcoma, or histiocytoma was generally < 3 AEs per 10,000 encounters (Table 4.4.2.1-4). ProHeart 6, whether administered alone or with a vaccine, was associated with a slight (~ 2 per 10,000 encounters), but higher rate of mast-cell tumors compared with administration of vaccines alone or any of the monthly heartworm preventatives. The rate of mast cell tumors per 10,000 days at risk was similarly elevated in dogs receiving ProHeart 6 compared with those receiving a vaccine or any one of the monthly heartworm preventative drugs. However, compared with dogs that had received 5 doses of ProHeart 6, there was no statistically significant difference in the age-adjusted risk of developing mast cell tumor for dogs that had received 1, 2, 3, or 4 doses of ProHeart 6 from Banfield veterinarians. That is, all of the odds ratios included 1.0. There was also no apparent dose-response relationship

between ProHeart 6 dose number and mast cell tumor risk. However, it is not known if any dogs had previously received ProHeart 6 from a non-Banfield veterinarian.

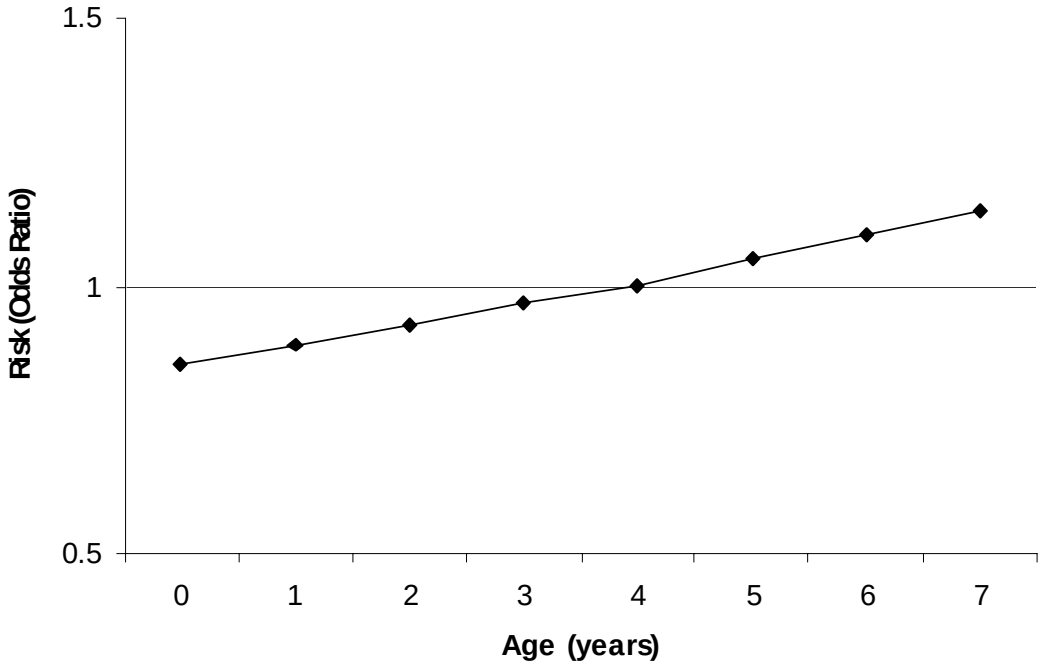
Table 4.4.2.1-4. Potential Cancer-related Adverse Events per 10,000 by Treatment Group

Treatment Category					Mast Cell Tumor		Lymphosarcoma		Histiocytoma	
PH6	HW Prev 1	HW Prev 2	Any Vaccine	N	N	Rate	N	Rate	N	Rate
				4,144,984	1,285	3.1	1,021	2.5	1,434	3.5
			Y	1,489,032	172	1.2	36	0.2	359	2.4
		Y		6,430	0	0.0	0	0.0	0	0.0
		Y	Y	11,975	0	0.0	0	0.0	2	1.7
	Y			164,951	10	0.6	12	0.7	45	2.7
	Y		Y	246,131	13	0.5	0	0.0	62	2.5
Y				252,590	54	2.1	13	0.5	89	3.5
Y			Y	483,064	90	1.9	14	0.3	101	2.1
Any				6,800,061	1,624	2.4	1,096	1.6	2,092	3.1

4.4.2.2 Multivariate Analyses

To control for potential confounding effects and to identify interactions between the variables, multivariate logistic regression models were constructed that included the following variables: heartworm preventative, vaccine, age, weight, non-steroidal anti-inflammatory (NSAID), steroid, and ProHeart 6 dose number. In the liver disease model, steroid use was associated with a 25% increased risk while ProHeart 6 was associated with a 15% reduction in risk. Each additional dose of ProHeart 6 was associated with an 8% reduction in liver disease risk. There was evidence of a strong interaction (effect modification) between age and ProHeart 6. Upon further examination of the relationship between ProHeart 6 dose number and age, the risk of liver disease increased with age, regardless of the exposure group. Using the best-fit equation generated from the logistic regression model, the relationship between the risk of liver disease associated with ProHeart 6 use was graphed as a function of age (Figure 4.4.2.2-1). ProHeart 6 administration was associated with a decreased risk of liver disease in dogs < 4 years of age, whereas there was an increased risk in dogs > 4 years of age.

Figure 4.4.2.2-1. Risk of Liver Disease by Age for ProHeart 6



Risk (1yr old) = 0.854*1.043 = 0.89; Risk(7yr old) = 0.853*1.043**7 = 1.14
Risk (age yrs) = OR(Proheart6) * OR(Proheart6*age interaction)**age

In the allergic reaction multivariate logistic regression model, ProHeart 6, Heartworm Preventative 1, vaccines, NSAIDS, and glucocorticoids, were all associated with an increased risk of allergic reactions; vaccine had the greatest affect. However, each additional dose of ProHeart 6 did not further increase the risk of allergic events.

Multivariate models for the risk of cancer indicated that ProHeart 6 was associated with a modest increase in the risk of mast cell tumor. It is not clear why NSAIDs were associated with a 423% increase in the risk of mast-cell tumor. Glucocorticoids were associated with a 182% increased risk of lymphosarcoma, probably because glucocorticoids are used as a treatment for this cancer. None of the heartworm preventatives were associated with the risk of histiocytoma.
In the multivariate model for the risk of death, only Heartworm Preventative 1 was associated with an increased risk (23%), whereas ProHeart 6 was associated with a 71% reduction in risk of

death. There was no positive relationship between the number of ProHeart 6 doses administered and risk of death (odds ratio = 0.910).

4.4.2.3 Temporal Trends in Event Rates

The rate of cancer, deaths, liver disease, and allergic reactions, were evaluated by quarter of the year for ProHeart 6 and Heartworm Preventative 1, both with and without vaccines, to determine if AEs associated with ProHeart 6 increased during the 3rd quarter of 2004, as suggested by the FDA. No evidence was found to suggest that AE rates associated with either ProHeart 6 or Heartworm Preventative 1, when used without a vaccine, increased in the 3rd quarter of 2004. In contrast, there was an increase in the 3rd quarter of 2004 in the rate of allergic events associated with either ProHeart 6 or Heartworm Preventative 1 when administered with a vaccine. Therefore, the increased rate of allergic events may be explained by 1 or more vaccines new to the market in mid-2004 that were used by Banfield veterinarians and that caused an increased rate of allergic reactions when administered with either ProHeart 6 or Heartworm Preventative 1 compared with administration of either of these heartworm preventatives without a vaccine.

4.4.3 Discussion

The results of these analyses involving almost 7 million dog encounters at Banfield clinics in the US did not reveal any clinically significant increase attributed to ProHeart 6 use in the risk of liver-related AEs, neurological disease, ocular disease, immune-mediated disease, cardiovascular disease, anaphylaxis, inflammatory bowel disease, or death, when compared with 2 commonly used monthly oral heartworm preventatives. While ProHeart 6 was associated with an increased rate of some liver-related AEs in the univariate analyses, this increased rate was not found in either the days at risk or in the multivariate analysis. In contrast, ProHeart 6, monthly heartworm preventatives, and vaccines were all associated with a clinically significant increased risk of allergic reactions in both univariate and multivariate analyses, especially during the first few days post-exposure.

The only potential AE studied that was independently associated with an increased risk following ProHeart 6 use in dogs was mast cell tumor. However, the absolute magnitude of the risk of mast-cell tumor associated with ProHeart 6 alone (2.1 events per 10,000 doses administered) or ProHeart 6 plus vaccine (1.9 events per 10,000 doses administered) was small. In addition, there was no statistically significant dose response relationship between the risk of

mast cell tumor and the cumulative number of doses of ProHeart 6 that a dog had received. Compared with the rate of mast cell tumor in dogs that had received a vaccine alone (1.2 events per 10,000 doses administered), the rate of mast cell tumor in dogs that had received ProHeart 6 plus a vaccine (1.9 events per 10,000 doses administered) does not appear to be clinically significant. There is no known mechanism that would explain how ProHeart 6 or any other heartworm preventative induces or promotes mast cell tumor in dogs, especially within 30 days of its administration.

Evidence was presented to show that the number of AEs associated with ProHeart 6 use increased in the 3rd quarter of 2004 compared with previous quarters. However, this increase primarily involved allergic reactions, was also observed in dogs that had received monthly oral heartworm preventatives, and was restricted to dogs that simultaneously received a vaccine. These findings suggest that any observed increase in adverse events associated with heartworm preventatives in the 3rd quarter of 2004, were likely due to one or more vaccines that were given with heartworm preventative drugs. Since nearly two-thirds of the heartworm preventative drugs studied were administered simultaneously or in close proximity with a vaccine, and since vaccines are generally associated with a higher rate of allergic reactions than are heartworm preventative drugs, AE reports involving administration of ProHeart 6 or other heartworm preventative drugs must be interpreted cautiously and take into account a dog's vaccine history.

4.4.4 Conclusions

In summary, the safety profile of ProHeart 6 appears similar to that of 2 commonly used monthly oral heartworm preventatives. The results of this study provide no support for the withdrawal of ProHeart 6 from the veterinary market. Lack of compliance is recognized as a common reason for lack of efficacy for both human and veterinary drugs. Since the likelihood of exposure of dogs to *Dirofiliaria immitis* infected mosquitoes is common during many months of the year, drugs are needed that prevent heartworm infection for an extended period of time, thus obviating the need for owners to remember to retreat their dog. The results of this study indicate the risks associated with ProHeart 6 use are few and similar to that for monthly oral heartworm preventatives that only offer protection from heartworm infection for a period of 30 days.

4.5 Field Efficacy

4.5.1 Overview

While the clinical efficacy of ProHeart 6 has been established in controlled laboratory and field studies, evaluation of efficacy under conditions of commercial use after launch can be complicated by many confounding factors. These include heartworm testing protocols, the age at which dogs commence a ProHeart 6 program, and timing of change from other prophylaxis to ProHeart 6.

Products for heartworm prophylaxis have been available in veterinary medicine for decades, initially as daily oral treatments; later, a range of monthly oral and topical treatments were introduced with ivermectin, selamectin, or milbemycin oxime as the active ingredient.[2,3,5] Some breeds of dogs exhibit toxic signs at doses of ivermectin and/or milbemycin oxime that are well tolerated in most other breeds, and a genetic basis for this sensitivity has been identified.[10,53,54,55,56,57,58,59] Moxidectin can be used safely in these breeds based on FDAH clinical studies.

4.5.2 Compliance

A high level of efficacy is found with these therapeutic options in controlled studies. However, under field conditions, an increasing incidence of heartworm infections in dogs in the US in the 1990s was reported with approximately 240,000 dogs testing heartworm positive in 2001. Surveys of dog owners have shown that compliance (ie, reliable administration of monthly treatments by owners) is problematical. Despite reminder systems such as calendar stickers, a survey in the US in 2000 found that >80% of participants had failed on multiple occasions to give their dogs the monthly preventative on the indicated day, and about one-third of participants completely missed the monthly dosage. Approximately one-fifth of the participants had missed giving their dogs the monthly oral heartworm preventative and then stopped altogether.[60] Another survey conducted in 2001 found that only 55% of dog-owning households in the US were using heartworm prevention, which was down from a high of 66% in 1998, despite heartworm having been diagnosed in all states.[61] Similarly, Yabsley et al. reported an increased prevalence of heartworm in shelter dogs in South Carolina in 1999-2000 (12.7%) versus 1991-1992 (8.7%).[62] Compliance with dosing schedules is a limiting factor in the control of heartworm infections in the dog population.

Several years ago, Cummings et al. demonstrated that clinic compliance failure is generally higher than predicted by individual hospitals before their records are examined.[63] A veterinary practice survey conducted by Fort Dodge veterinary technicians at Michigan State in 2004 showed a similar picture.[64] Compliance was better with ProHeart 6 than with the other heartworm preventatives studied. Average compliance was 58% for ProHeart 6 (11 clinics), 45% for Heartgard Plus (the most widely used preventative)(16 clinics), 50% for Sentinel (6 clinics), and 37% for Interceptor (3 clinics).

Moxidectin is a poor substrate for P glycoprotein compared with other macrocyclic lactones.[59,65,66] It can be used safely in ivermectin sensitive Collies. It is effective in the face of ivermectin resistance in nematodes. Additionally, recent work has demonstrated that with use of moxidectin, unlike some other compounds, animals are not more susceptible to infection with filariid parasites after product withdrawal.[67] This provides an additional safety factor, if owners are late in returning for treatment. Moxidectin has retroactive activity against *D. immitis* larval stages for 3 months, again providing a safety factor, if owners are late in returning for treatment.

4.5.3 Field Efficacy from 2001 to 2004

Field experience with ProHeart 6 in the US is limited to the time period from June 2001 to August 2004. There is a time-lag between infection and the first possible heartworm diagnosis of minimally 5 to 6 months. Therefore, the field evaluation of ProHeart 6 efficacy essentially spans the years 2002 to mid 2004. Proving "lack of efficacy" of any heartworm preventative product in client-owned dogs is inherently difficult, unless the dog is started on a prevention program at 6- to 8-weeks of age, remains on the product year-round for life and clinic records indicate the owner acquired the recommended number of doses. With orally or topically administered products, one is seldom confident the owner properly administered all of the doses. Owner compliance is greatly improved with ProHeart 6, but switching products tends to complicate the picture. To prove with reasonable confidence that a new product is completely effective, the dog must be tested prior to switching products and within 4 months later. If the dog is heartworm positive during this 4-month period, the original product failed. If the dog is positive during the next few months, it is virtually impossible to determine whether the dog was infected before or after switching products. Voluntary reports of product inefficacy to FDA are summarized in Table 4.5.3-1.

Table 4.5.3-1. Voluntary Reports of Product Inefficacy Against Larval Heartworm

Active	2001	2002	2003	2003 Market Share
IVM	16	108	13	6%
IVM+PYR	21	195	137	37%
MILB	0	0	748	21%
MILB+LUF	0	0	347	8%
SEL	16	1162	888	4%
ProHeart 6	0*	70	196	24%

* indicates year of launch

IVM = ivermectin; PYR = pyrantel; MILB = milbemycin oxime; LUF = lufenuron; SEL = selamectin

The 2003 market share information is indicative of the numbers of dogs treated and provides context when interpreting the significance of the number of reports. When ProHeart 6 is compared with the market leaders the adjusted efficacy rates for 2003 (when market share information is available) are equivalent or lower than ivermectin plus pyrantel, or milbemycin.

The overall reporting rate for ProHeart 6 is low at 1 report per 41,000 doses through marketing year 2003. Further, only 10% have a history that justifies classification as a potential efficacy concern. The majority are thought to be associated with a misunderstanding of the heartworm life cycle in relation to testing procedures and limitations. When potential dosing errors, administration errors, and mixing and storage errors are considered, the low reporting rate of failures in efficacy appears to be within acceptable parameters.

FDAH guarantees the efficacy of ProHeart 6 as a preventative for heartworm disease. This program encourages veterinarians to contact the company about potential concerns related to efficacy. When received, these reports are submitted to the FDA unfiltered. Based on unfiltered reports and doses sold, regardless of whether FDA or FDAH reports are considered, ProHeart 6 delivers a high level of efficacy under field use conditions.

4.5.4 Tuskeegee University Experience with ProHeart 6

Tuskegee University is located in Macon County, in eastern Alabama. It is a teaching hospital and unlike some universities which are predominantly referral centers, Tuskegee operates largely as a community practice. Most pets in the area receive their primary care at this hospital, so it is reflective of the population of dogs receiving preventative care, rather than specialist or tertiary care levels.

4.5.4.1 Retrospective Study: Tuskegee University School of Veterinary Medicine

The effectiveness of heartworm preventatives in this endemic area was studied during the period of 2000-2004 (unpublished data).[68] In 2001, 97.7% of the dogs on a heartworm preventative received an oral product, while 1.0% received ProHeart 6. By 2004, 82.9% received an oral product and 17.1% received ProHeart 6. During this time, the percentage of heartworm-positive dogs dropped from 14.0% (of 898) to 5.8% (of 833), and the percentage of dogs treated with Immiticide dropped from 5.2 to 3.0. More importantly, at no time was any dog receiving ProHeart 6 diagnosed as heartworm-positive, while dogs on the oral products accounted for 15.8%, 29.4%, and 50.0% of the dogs that received a first, second, or third Immiticide treatment, respectively. This study also reviewed owner compliance in a limited number of dogs that received Immiticide treatment. Of the 100 dogs studied, 61.5% of the dogs receiving ProHeart 6 were up-to-date on purchasing their heartworm product, while only 17.2% of those on oral products were compliant. Furthermore, it is known that 100% of the dogs on ProHeart 6 received their full treatment, whereas the number of oral doses administered is not known.

While this study represents a limited number of dogs, it supports the position that ProHeart 6 is highly effective in preventing heartworm infection, and along with increased owner compliance, has led to a decrease in the number of dogs requiring Immiticide treatment.

These results indicate that ProHeart 6 introduction into the canine heartworm preventative market is helping to achieve the desired goals. By overcoming the problems with owner compliance with monthly dosing, ProHeart 6 not only protects the individual dog to which it is administered, but also provides the veterinary professional with an important medicine to reduce the prevalence of heartworm in the canine population.

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6.0 APPENDICES

APPENDIX 6.1

MOXIDECTIN OVERVIEW

Pharmacology – Mechanism of Action

The mechanism of action of moxidectin is multifaceted and continues to be evaluated in laboratory studies. Moxidectin has been shown to have activity at the γ-aminobutyric acid (GABA)-A receptor-chloride channel complex resulting in an influx of chloride ions and hyperpolarization of cell membranes[1]. This hyperpolarization causes the nerve fibers to be less excitatory and results in paralysis and death of the parasitic organism. The specificity of moxidectin for the parasite versus the mammalian host results from 1) this compound having low affinity for mammalian GABA-gated chloride channels[2], and 2) the observation that GABA-containing neurons and receptors are found in mammals in the central nervous system, whereas in arthropods and nematodes these are found in the neuromuscular junctions of the peripheral nervous system and thus are more accessible to a blood-borne therapeutic. Another proposed mechanism of action for moxidectin is through activity at glutamate-gated chloride ion channels also resulting in paralysis and death of the organisms[3,4].

In dogs, the approved, oral monthly dosage of moxidectin for prevention of heartworm is 3 μg/kg; the approved subcutaneous (sc) dosage of moxidectin as ProHeart 6 is 0.17 mg/kg administered every 6 months.

Pharmacokinetics and Drug Metabolism

A series of studies was conducted in various animal species to characterize the absorption, distribution, metabolism, and excretion of moxidectin after oral administration. In rats, moxidectin was absorbed at a moderate rate, with a mean time to peak concentration (t_{max}) of 4.8 hours[5]. The bioavailability of moxidectin was moderate at 19% and the apparent terminal half-life ($t_{1/2}$) was long (22.9 to 44.6 hours). After intravenous (IV) administration in rats, the clearance of moxidectin was low and the steady-state volume of distribution (Vd_{ss}) was high, indicating that the compound is widely distributed to tissues. In beagle dogs after an oral dose of 90 μg/kg moxidectin in tablet form, the peak concentration (C_{max}) was 29.8 ng/mL with a t_{max} of

8 hours and a serum half-life of 8.1 days[6]. A single sc injection of the approved dosage of 0.17 mg/kg moxidectin as ProHeart 6 to beagle dogs resulted in a serum C_{max} of 5.1 ng/mL, a t_{max} of 7 to10 days, an $AUC_{0-\infty}$ of 217 ng•day/mL, and a half-life of approximately 35 days[6]. Moxidectin did not accumulate in the serum of dogs after injection with ProHeart 6 once every 6 months for a total of 6 injections[6]. A recent study of the administration of moxidectin once in the diet to female dogs at 45 ppm (corresponding to approximately 1 mg/kg as used in the 1-year toxicology study) resulted in a Cmax, $AUC_{0-\infty}$, and half-life values of 290 ng/mL, 678 ng•day/mL and 8.3 days, respectively. Administration in the diet at this same level for 21 days to dogs resulted in a plasma concentration of 278 ng/mL approximately 24 hours after the previous feeding (ie, trough level). The previous 2 ongoing diet pharmacokinetic studies in the dog, and an ongoing 28-day pharmacokinetic study in rats by the diet route were initiated because of FDA concerns regarding the safety of ProHeart 6, and to document the high degree of systemic exposure to moxidectin achieved in the toxicology studies conducted by the diet route.

After oral administration in rats, the major site of moxidectin distribution was fat[5]. The $t_{1/2}$ in fat was 11.5 days. Moxidectin represented the major component of total radioactivity in tissues and feces, and was primarily eliminated through feces (60% to 91% of the recovered dose over 7 days)[5]. Studies in other species such as cattle, sheep and horses have confirmed the distribution of moxidectin primarily to fat and the fecal route of excretion[7]. Six (6) metabolites were isolated from rat liver and fecal samples, none of which accounted for more than 10% of the radioactivity in tissue samples collected from animals 7 days after dosing[5]. Therefore, these metabolites are not of toxicologic concern because of the low levels observed. Similarly limited metabolism was noted in cattle, sheep and horses[7] and in rat and human liver microsomes[8] where the metabolites were characterized as hydroxylations at various positions.

In human liver microsomes, there was no significant inhibition of selected cytochrome P450 (CYP) enzyme activities (CYP2A6, CYP2C8, CYP2C19, CYP2D6, and CYP3A4) at the highest substrate concentration used (100 μM)[9]. Human CYP enzymes were used in this study since they are the best characterized. Based on plasma concentrations at efficacious doses and the high substrate concentrations, clinical metabolic drug-drug interactions for all the CYPs tested are unlikely to occur.

P-glycoproteins (P-gps) are transmembrane proteins which transport a wide variety of endogenous and exogenous molecules across cell membranes. Moxidectin, as do other macrocyclic lactones, acts as a substrate for P-gps[10]. This mechanism has been found to be of clinical importance in the development of nematode resistance to ivermectin and plays a significant role in breed sensitivity[11]. A mutation in the P-gp gene of ivermectin-sensitive Collie dogs has been shown to be responsible for ivermectin-induced CNS toxicity[12]. Moxidectin, however, was well-tolerated by these ivermectin-sensitive dogs. Moxidectin transport, therefore, is less dependent on P-gp and subsequent toxicity is less likely to be altered by factors which alter P-gp activity.

Toxicology

The toxicologic profile of moxidectin administered by the oral route has been well established. This profile is relevant to other routes of administration because of limited metabolism of moxidectin in the body and an understanding of its pharmacokinetics by different routes of administration. The toxicologic program for moxidectin was reviewed during the 45th meeting of the Joint Expert Committee on Food Additives[13] and was subsequently published[7]. In addition, this toxicology program was deemed sufficient by regulatory authorities to proceed with Phase 1 trials of oral moxidectin in normal, human volunteers in preparation for efficacy studies in people in countries where onchocerciasis is endemic. The toxicology studies were conducted in accordance with Good Laboratory Practice (GLP) regulations.

In Vitro Side-Effect Profiling

Moxidectin and moxidectin microspheres (as present in ProHeart 6) were recently tested in vitro for binding activity at 64 different biological receptors[14], based on some of the safety concerns raised by the FDA. This assay is commonly used in drug discovery and development to identify any ancillary pharmacologic activities of a molecule which may result in undesirable biological effects. A final concentration of 10 ng/mL moxidectin was tested, which is approximately two-fold the average C_{max} value in serum of dogs after a subcutaneous injection of ProHeart 6 at the clinical dosage of 0.17 mg/kg. The receptors tested included those for neurotransmitters and neurotransmitter-related receptors, ion channels, steroids, second messengers, prostaglandins, growth factors/hormones, brain/gut peptides and enzymes. Moxidectin in either form did not significantly inhibit the binding of appropriate radio-ligands to these receptors, indicating a lack of binding activity for moxidectin. These results are consistent with the absence of undesirable pharmacologic and toxic effects of moxidectin in animal studies at plasma levels many fold those required for efficacy.

Single-Dose Studies

Single-dose toxicology studies of moxidectin were conducted to assess effects after a single administration of large doses in the event of accidental overdose and to assist in selection of dose levels for subsequent repeat-dose toxicology studies. In single-dose toxicity studies of mice and rats given moxidectin orally, the median lethal dosage (LD_{50}) values were 118 mg/kg in male mice[15], 42 to 78 mg/kg in female mice[16,17], and 122 and 97 mg/kg in male and female rats[18], respectively. After a single sc dose, LD_{50} values were 285 and 247 mg/kg in male and female mice[19], respectively, and > 640 mg/kg in rats[20]. Common clinical signs in these studies were decreased activity, tremors, and prostration. The decreased toxicity with sc dosing is likely the result of a lower C_{max} and daily exposure, albeit of longer duration, as compared to oral gavage dosing. These studies demonstrate a large margin of safety for ProHeart 6 in which the approved sc dose in the dog is 0.17 mg moxidectin/kg, less than 3700-fold the lethal sc dose in the rat. Field results from the accidental and sometimes intentional overexposure of dogs orally to moxidectin likewise demonstrate a large margin of safety. Oral overdoses occur when a product intended for use in horses is administered to dogs. In these cases, the dogs can receive up to a 63-fold higher dose orally (ie, 10.7 mg/kg) than the 0.17 mg moxidectin/kg when given sc as ProHeart 6, but in most cases the actual dose is not known. From 1997 to 2004, FDAH received approximately 250 such cases. In all cases the events were characterized by a wide range of neurological symptoms. In those cases where FDAH was informed, the dogs had typical hematology or serum panels. In 90% of these cases, the animals fully recovered whereas the remainder died. These cases demonstrate that neurological symptoms induced by high, oral doses of moxidectin in dogs are typically survivable without any nonneurologic toxic effects.

Repeat-Dose Studies

Repeat-dose oral (diet) toxicity was evaluated to assess more long-term consequences of repeated, daily oral exposure to moxidectin. The objective of these studies was to dose the animals high enough to identify potential toxic effects, and to include lower doses so as to assess a dose-response relationship and identify a dose without significant adverse effects (i.e., No-Observed-Adverse-Effect-Level; NOAEL).

The following studies were conducted: 4-week studies in mice, rats, and dogs; 13-week studies in rats and dogs, and a 1-year study in dogs. Moxidectin was administered in the diet providing for a continuous exposure for the duration of each study, which is a significant exaggeration of the exposure of dogs to one sc dose of 0.17 mg moxidectin/kg as ProHeart 6 every 6 months.

Evaluations consisted of mortality, clinical observations, body weight, food consumption, hematology, clinical chemistry (except mice), organ weights, and macroscopic and microscopic examinations of organs and tissues. Ophthalmic examinations and urinalysis were also included in the dog studies.

Mice

In the 4-week study in mice, doses in the diet were 33.7, 75, 100, 125, and 150 ppm[21]. Mortality occurred at ≥ 75 ppm. Microscopic examination did not reveal the cause of death in these animals. Clinical observations were tremors, hypersensitivity to touch, and urine-stained fur at ≥ 75 ppm. Body weights and body-weight gains were decreased at ≥ 100 ppm. There were no other compound-related effects. Based on mortality, the no-observed-adverse-effect-level (NOAEL) was 33.7 ppm (6.9 mg/kg/day).

Rats

In the 4-week study in rats, doses in the diet were 100, 200, 400, and 600 ppm[22]. Mortality occurred at ≥ 200 ppm and resulted from anorexia based on microscopic examination. Clinical observations included ataxia, tremors, salivation, piloerection, and diuresis at ≥ 200 ppm, as well as decreased body weight, body-weight gain, and food consumption. There were no other compound-related findings in rats that survived the study. Based on mortality, the NOAEL was 100 ppm (12.2 mg/kg/day).

In the 90-day study in rats at diet doses of 25, 50, 100 and 150 ppm, mortality occurred for 3 females at the highest dose, 150 ppm[23]. Microscopic examination did not reveal the cause of death for these animals. Clinical observations included hypersensitivity to touch at 100 ppm, and lethargy, anorexia, aggressive behavior, loss of righting reflex, ataxia, tremors, and urine-stained coat at 150 ppm. Body weight and body-weight gain were decreased in males at 100 ppm or more and in females at 150 ppm. Food consumption was decreased in males and females at 150 ppm. Adrenal gland and kidney weights were increased in females at ≥ 100 ppm, but there were no compound-related macroscopic or microscopic findings in these or any other organs or tissues. The NOAEL was 50 ppm (3.9 mg/kg/day).

Dogs

In the repeat-dose toxicity studies in beagle dogs, ophthalmic examinations and urinalysis were also included for evaluation of potential toxic effects.

In the 4-week study, there was no mortality at doses in the diet of 20, 80 and 160 ppm[24]. The dose of 160 ppm was toxic, based on debilitating clinical observations, and the dose was reduced to 50 ppm. Clinical observations included tremors, languid appearance, ataxia, emesis, and mydriasis at $\geq$ 80 ppm. Testes weights were decreased at $\geq$ 80 ppm. Microscopic findings were decreased spermatogenic activity at $\geq$ 80 ppm and decreased colloid in the thyroid gland in males at 80 ppm. These findings may have been related to variations in age of maturation (study dogs were 5 to 6 months old at study termination) and were not seen in the 1-year toxicity study in dogs. Based on the testicular findings, the NOAEL in this 4-week study was 20 ppm (0.8 mg/kg/day).

In the 90-day toxicity study in dogs at diet doses of 10, 30 and 60 ppm, there was no mortality and clinical observations included lacrimation at $\geq$ 10 ppm, languid appearance and tremors at 10 and 60 ppm, thin appearance at $\geq$ 30 ppm, and slight salivation and slight ataxia at 60 ppm[25]. A 60 ppm, alanine aminotransferase (ALT) was increased in 1 male and 1 female, and serum alkaline phosphatase was increased in 3 males and 3 females compared with pretreatment and/or control values (increases were small in magnitude and not toxicologically significant). However, there were no compound-related macroscopic or microscopic changes in the liver or any other tissues or organs. Based on the absence of toxicologically significant findings at any tested concentration, the NOAEL in this 90-day study was 60 ppm (1.6 mg/kg/day).
In the 1-year toxicity study in dogs at diet doses of 10, 20 and 45 ppm, there was no mortality[26]. Decreased ovarian weights were observed at 45 ppm; however, the absolute weights were within reference or historic control ranges and the decreases were not considered to be biologically important. There were no compound-related effects on clinical chemistry or hematology parameters, and no compound-related microscopic findings. Based on the absence of toxicologically significant findings at any tested concentration, the NOAEL in this study was 45 ppm (1.1 mg/kg/day).

Interim analysis at day 21 in an ongoing 28-day diet pharmacokinetic study of moxidectin in dogs at a concentration of 45 ppm in feed (approximately 1 mg/kg, the NOAEL in the 1-year dog toxicity study) revealed a serum concentration of 278.5 ng/mL 24 hours after the preceding dose in feed (ie, trough level). This concentration is not yet at steady state, but would correspond to a daily AUC of 278.5 ng•d/mL which would represent the minimal daily exposure to moxidectin

for most of the duration of the 1-year toxicology study in dogs, or an AUC of 50826 ng.day/mL over a 6-month period, the retreatment interval for ProHeart 6.. This value is approximately 234-fold the AUC $_{0-\infty}$ (217 ng•d/mL) observed for moxidectin after a single sc dose in dogs of ProHeart 6.

Carcinogenicity Studies

Mice

A 2-year carcinogenicity study was conducted in male and female mice at diet doses of 15, 30, and 60 ppm (lowered to 50 ppm due to high mortality at week 9)[27]. Mortality was increased in females at doses of 60/50 ppm during the last 13 weeks of the study. There were no compound-related findings in hematology values, organ weights, or at macroscopic or microscopic examination. There was no evidence of moxidectin-related target-organ toxicity or tumorigenicity.

Rats

A 2-year carcinogenicity study was conducted in male and female rats at diet doses of 15, 60 and 120 ppm (lowered to 100 ppm due to high mortality in females at week 8)[28]. There were no compound-related findings for hematology values, organ weights or at macroscopic or microscopic examination; there was no evidence of moxidectin-related target-organ toxicity or tumorigenicity.

Reproductive and Developmental Toxicity Studies

Reproductive toxicity was evaluated in developmental studies of rats[29] and rabbits[30] dosed orally by gavage and in pilot[31] and definitive[32] multigeneration diet studies. Maternal toxicity was evident at ≥10 mg/kg/day in the rat developmental study, 125 ppm (calculated dosage averaging between 10.9 and 12.0 mg/kg/day) in the rat pilot multigeneration study, and at ≥5 mg/kg/day in the rabbit developmental study; the toxicity consisted primarily of decreased body weight and/or food consumption. At maternally toxic dosages (≥ 10 mg/kg/day) in the rat developmental study only, there were statistically significant increases in the number of fetuses with malformations and/or variations, largely reflective of increases in cleft palate and reversible delays in ossification. There was decreased fetal and/or pup survival at 10 mg/kg/day in the rabbit developmental study and at doses of ≥10 ppm (calculated maternal dosage of ≥ 0.8 mg/kg/day) in the rat pilot 1-generation and 3-generation studies. The reproductive NOAELs were

5 mg/kg/day in both the rat and rabbit developmental studies and was 5 ppm (0.4 mg/kg/day) in the rat 3-generation study. Therefore, moxidectin was not considered teratogenic in these species. This conclusion is consistent with that of the FDA Center for Veterinary Medicine, which concluded that moxidectin is neither a selective developmental toxicant nor a teratogen in rats or rabbits.

Genotoxicity Studies

Moxidectin was tested for genotoxicity in 4 in vitro and 2 in vivo universally recognized test systems. These assays assessed the ability of moxidectin to caus gene mutations, chromosome damage, and increased DNA repair which may be related to the carcinogenic potential of the test article. Moxidectin was negative in the following assays for genotoxicity: bacterial reverse mutation assay in *Salmonella typhimurium* (strains TA98, TA100, TA1535, TA1537, and TA1538) and *Escherichia coli* (WP-2 uvrA-)[33]; mammalian cell mouse lymphoma (L5178Y thymidine kinase) assay[34]; chromosome aberration assay in Chinese hamster ovary cells[35]; unscheduled DNA synthesis assay in primary rat hepatocytes[36]; in vivo micronucleus assay in mouse bone marrow cells[37]; and the in vivo chromosome aberration assay in rat bone marrow cells[38]. The in vivo studies were conducted using the oral route of administration. The results from these studies indicate that moxidectin is not a genotoxic compound.

Experience with Oral Moxidectin in Human Volunteers

A study in healthy, male volunteers was conducted to assess the pharmacokinetics and safety of moxidectin given orally as part of the development of this compound for treatment of onchocerciasis in humans[39]. A total of 37 subjects in this study were treated with single doses of 3 mg to 36 mg (approximately 50 μg/kg to 600 μg/kg). The $t_{1/2}$ ranged from 19.9 to 37.4 days, the C_{max} at 36 mg was 296 ng/mL, and the distribution of moxidectin was extensive, as indicated by a large apparent volume of distribution. The C_{max} observed for humans dosed orally with 36 mg moxidectin was 59-fold the C_{max} observed in dogs dosed sc with the clinical dosage of 0.17 mg/kg moxidectin as ProHeart 6, and exposure (based on AUC) was approximately 6-fold the exposure in dogs administered ProHeart 6.

There was no significant relationship between the overall number of adverse events and the dose of moxidectin administered. Safety assessments indicated that moxidectin was safe and well tolerated, with a slightly higher incidence of transient, mild, and moderate central nervous system adverse events (dizziness and somnolence) as compared to placebo. There were no clinically significant changes in vital signs, clinical chemistries, physical examinations or

electrocardiograms. The conclusion was that moxidectin was safe and well tolerated in humans after single, oral doses of 3 mg to 36 mg.

Conclusions

Moxidectin is a potent antiparasitic therapeutic that acts to paralyze susceptible organisms through activity at GABA- and glutamate-gated chloride ion channels. Moxidectin has a long half-life, a high volume of distribution (predominantly distributing to fat), shows little metabolism, and is excreted primarily in the feces. These characteristics of moxidectin appear constant across the mammalian species studied, including humans. In single-dose toxicity studies, the lethal sc dose in rats was more than 3700-fold the efficacious dose of moxidectin given as ProHeart 6 to dogs. In repeat-dose diet toxicity studies in mice,rats, and dogs of durations up to 2-years in mice and rats, and 1-year in dogs, no target organs of toxicity were identified. There were no significant adverse histologic or biochemical effects on any organ system including the cardiovascular, gastrointestinal, hepatobiliary, genitourinary and central nervous systems. There were no proliferative lesions identified in any tissue which may signal the development of neoplasia, and no increase in tumors in mice or rats. The toxicity of moxidectin manifested itself at high doses in clinical signs such as lethargy, ataxia, tremors and mortality (rodents only) with concomitant decreases in food consumption and body weight. Such clinical signs of hypoexcitation are expected and are consistent with an exaggerated pharmacologic effect of moxidectin mediated via the GABA-receptor. Moxidectin was not genotoxic or carcinogenic, and was without reproductive or developmental toxicity. The NOAEL in the rat 2-year study was 12.2 mg/kg/day; the NOAEL in the dog 1-year study was 1.1 mg/kg/day.

Based on ongoing pharmacokinetic studies in the dog, a single dose of moxidectin in the diet at approximately 1 mg/kg (NOAEL in the 1-year dog study), when extrapolated to infinity, results in an overall exposure over 3-fold that of a single SC dose of ProHeart 6. Repeated dosing at this level resulted in an estimated daily exposure at least 1.3-fold the total exposure observed after a single SC dose in dogs of ProHeart 6 (AUC_{1day} of 278.5 versus $AUC_{0-\infty}$ of 217 ng•days/mL, respectively). The total exposure over the duration of the 1-year study, therefore, would be approximately 365 times the daily AUC, or more than 450-fold a single SC dose of ProHeart 6. Moxidectin was also found safe in a human clinical trial after a single oral dose of

36 mg, which resulted in a C_{max} and AUC approximately 59- and 6-fold, respectively, that was observed in dogs dosed SC with the clinical dosage of 0.17 mg/kg as ProHeart 6.

Based on the toxicology studies of moxidectin where the dose, dosing duration, and resulting systemic exposure to moxidectin were significantly exaggerated without inducing significant adverse effects, the single, clinical sc administration of 0.17 mg moxidectin/kg as ProHeart 6 to dogs would be predicted to be without significant adverse effects.

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APPENDIX 6.2

BIOGRAPHICAL SKETCH

Name:	Title:	Birthdate:
Alexander de Lahunta	James Law Professor of Anatomy	3 December 1932

Education:

Cornell University	DVM	1958	Veterinary Medicine
Cornell University	PhD	1963	Veterinary Anatomy

Professional Experience:
Veterinary Practice 1958-1960: Concord N.H. Faculty member in Department of Anatomy, New York State College of Veterinary Medicine, Cornell University 1960 to date; Chairman - Department of Clinical Sciences 1977-1986; Chairman - Department of Anatomy 1986 to 1991; Consultant in clinical neurology to Teaching Hospital 1963 to date; Diplomat - Neurology Specialty - American College of Veterinary Internal Medicine.
James Law Professor of Anatomy, 1992.

Awards:
National Teaching Award - Basic Sciences: Student AVMA 1991
Norden Teaching Award - 1973, 1984, 1992, 2001
ACVIM Dr. Robert W. Kirk Distinguished Service Award, 2000
Honorary Member - American College of Veterinary Pathologists, 2002

Research:
Correlation of clinical neurological signs with specific anatomic locations of lesions in nervous system.
Establishment of reliable data to differentiate between the various diseases that affect the nervous system in the different species of domestic animal.
Recognize and publish new diseases of the nervous system.
Recognize diseases of the nervous system of domestic animals that are models for similar diseases in man.

Textbook Publications:
Evans, H.E. & de Lahunta, A. - Miller's Guide to the Dissection of the Dog, 5th edition. 2000, W.B. Saunders Co., Phil.

de Lahunta, A. - Veterinary Neuroanatomy and Clinical Neurology, 2nd. edition. 1983, W.B. Saunders Co., Phil.

Noden, D. and A. de Lahunta - The Embryology of Domestic Animals, Developmental Mechanisms and Malformations 1985, Williams & Wilkins, Baltimore.

de Lahunta, A. and R.E. Habel - Applied Veterinary Anatomy 1985, W.B. Saunders, Phil.

Summers, B.A., J.F. Cummings and A. de Lahunta – Veterinary Neuropathology 1995, Mosby, St. Louis

260 Publications in refereed journals.

January 3, 2005

Teaching Responsibilities:

Block I: Lectures, Tutor, Gross dissection labs, Radiology labs. (Sept – Nov)

Vet Med 521: Neuroanatomy and Clinical Neurology
Entirely my responsibility, first 8 weeks of spring semester.

Block V: Applied Anatomy taught throughout most of Block V throughout the latter half of the spring semester and all of the fall semester.
Neuropathology – 12 hours, last 3 weeks of fall semester.

Vet Med 606: Advanced Neurology taught twice, in both of the last two quarters of the spring semester.

Neuropathology Seminar: One hour per week all year. Primarily for pathology residents.

Clinical Neurology Rounds: 12 hours per week all year.

Consultant to the Teaching Hospital for neurology patients:
Examine patients daily, all year long. This is done early each morning and often attended by interested students.

I regularly receive nervous tissue from practitioners and pathologists for study or consultations and videotapes from practitioners and owners for study and diagnosis. My lab is set up for blocking these nervous tissues and photographing the lesions. Tissue sections are cut and stained by the histology lab in the pathology section.

I study these sections and report results to the contributor. This is a valuable source of material for the teaching program both at the DVM and resident level and has led to the discovery of many new disorders.

APPENDIX 6.3

REBAR, ALAN H., DVM, PhD, DACVP 1/05

EDUCATION

DVM, 1973, Purdue University
Ph.D. (Clinical Pathology), 1975, Purdue University

Internship/Residency (Pathology), 1976, Purdue University
Diplomate – American College of Veterinary Pathologists (Clinical Pathology), 1978

EMPLOYMENT

July 1996 – Present	Dean, School of Veterinary Medicine, Purdue University
July 1989 – June 1996	Associate Dean for Research, School of Veterinary Medicine, Purdue University
July 1995 – June 1996	Head, Veterinary Pathobiology, School of Veterinary Medicine, Purdue University
July 1993 – June 1995	Interim Department Head, Veterinary Pathobiology, School of Veterinary Medicine, Purdue University,
July 1987 – June 1995	Acting Director of Continuing Education, School of Veterinary Medicine, Purdue University
July 1987 – July 1989	Director of Research Programs Development, School of Veterinary Medicine, Purdue University
1986 – Present	Director, Veterinary Cytology Resource Center, Purdue University,
July 1983 – Present	Professor of Clinical Pathology, School of Veterinary Medicine, Purdue University
July 1979 – July 1987	Co-Director of Clinical Pathology Laboratory, School of Veterinary Medicine, Purdue University
July 1979 – June 1983	Associate Professor of Clinical Pathology, School of Veterinary Medicine, Purdue University
Sept. 1977 – July 1979	Clinical Pathologist, Lovelace Inhalation Toxicology Research Institute, Albuquerque, New Mexico
Jan. 1976 – Aug. 1977	Director of Clinical Pathology Laboratory, School of Veterinary Medicine, Purdue University

Jan. 1976 – Aug. 1977	Assistant Professor of Clinical Pathology, School of Veterinary Medicine, Purdue University
Sept. 1974 – Dec. 1975	NIH Postdoctoral Fellow. (Ultrastructural studies on cobalt and isoproterenol induced cardiomyopathy in swine.) School of Veterinary Medicine, Purdue University,
Sept. 1972 – Aug. 1974	Graduate Instructor, Veterinary Pathology, School of Veterinary Medicine, Purdue University
May 1973 – Sept. 1973	Staff Veterinarian, Colonial Oaks Animal Hospital, Gainesville, Florida

HONORS

Indiana Veterinarian of the Year Award. Presented by the Indiana Veterinary Medical Association, 2002.

The Waltham Award given in recognition of outstanding activities or contributions by a veterinarian that have resulted in the improvement of the well-being companion animals in the international veterinary community. Presented by the American Animal Hospital Association, 2001.

The Gaines Cycle Fido Award for outstanding contributions to small animal medicine and surgery. Presented by the American Animal Hospital Association, 1994.

Distinguished Alumnus Award, School of Veterinary Medicine, Purdue University, 1990.

REPRESENTATIVE PUBLICATIONS (more than 115 overall)

1. Herbert, R.A.,Stegelmeier, B.S., Gillett, **Rebar, A.H**., Carlton, W.W., Singh, G., and Hahn, F.F.: *Plutonium-induced proliferative lesions and pulmonary epithelial neoplasms in the rat: immunohistochemical and ultrastructural evidence for their origin from type II pneumocytes*. Vet Pathol 31(3):366-374, 1994.
2. Stegelmeier, B.L., Gillett, N.A., Hahn, F.F., **Rebar, A.H**., and Kelly, G.: *Expression of transforming growth factor alpha and epidermal growth factor receptor in rat lung neoplasms induced by plutonium-239.* Radiat-Res, Nov, 140(2):191-8, 1994.
3. Lipscomb, T.P., Harris, R.K., **Rebar, A.H.**, Ballachey, B.E., and Haebler, R.J.: *Pathology of Sea Otters.* In: Marine Mammals and the Exxon Valdez. Academic Press, San Diego, CA. 1994.
4. Reagan, W.J. and **Rebar, A.H**., *Platelet Dysfunction.* In: Textbook of Veterinary Internal Medicine. W.B. Saunders Company, Philadelphia, PA. 1994.
5. Skowronek, L.A., LaFranco, L., Stone-Marschat, M.A., Burrage, T.G., **Rebar, A.H.**, and Laegreid, W.W.. *Clinical Pathology and Hemostatic Abnormalities in Experimental African Horsesickness*. Vet Pathol 32:112-121, 1995.

6. **Rebar, A.H.,** Lipscomb, T.P., Harris, R.K., and Ballachey, B.E. *Clinical and Clinical Laboratory Correlates in Sea Otters Dying Acutely in Rehabilitation Centers Following the Exxon Valdez Oil Spill*. Vet Pathol 32(4):346-350, 1995.
7. **Rebar, A.H.**, Metzger, F.: The Veterinary CE Advisor--*Clinical pathology for small-animal practitioners: Interpreting the hemogram*. Veterinary Medicine 90(6) (Suppl.):1-12, 1995.
8. Snipes, M.B., Barnett, Harkema, J.R., Hotchkiss, J.A., **Rebar, A.H.**, Reddick, L.J.: *Specific Biological Effects of an Anti-Rat PMN Antiserum Intraperitoneally Injected into F344/N Rats*. Vet Clin Pathol 24(1), 11-17. 1995.
9. **Rebar, A. H.**, Thrall, M. A.: *Blood Film Evaluation: Cytology of Circulating Blood Cells.* Vet Tech 16(9), 578-586, 607, 1995.
10. **Rebar, A.H.**, Metzger, F.: The Veterinary CE Advisor--*Clinical Pathology for Small-animal Practitioners: Profiling the Urinary System*. Vet Med 90(11) (Suppl.):1-16; 1995.
11. Christian, J.A., **Rebar, A.H.**, Boon, G.D., Low, P.S. *Methodological considerations for the use of canine in vivo aged biotinylated erythrocytes to study RBC senescence.* Experimental Hematology 24(1):82-8, 1996 Jan.
12. **Rebar, A.H.**, Metzger, , F.: The Veterinary CE Advisor--*Clinical Pathology for Small-animal Practitioners: Laboratory Evaluation of the Liver.* Vet Med 91(9) (Suppl.):1-12;1996
13. **Rebar, A.H.**, Section Editor, *Hematology and Immunology*. In: The 5 Minute Veterinary Consult, Tilley, L., and Smith, F., ed., Williams and Wilkins, Media, PA. 1997.
14. **Rebar, A.H.,** *Metabolic Anemias (Anemias with Spiculated Red Cells)*. In: The 5 Minute Veterinary Consult, Tilley, L., and Smith, F., ed., Williams and Wilkins, Media, PA. 1997.
15. Christian, J.A., **Rebar, A.H.**, *Anemia, Regenerative*. In: The 5 Minute Veterinary Consult, Tilley, L., and Smith, F., ed., Williams and Wilkins, Media, PA. 1997.
16. **Rebar, A.H.**, *Anemia, Nuclear Maturation Defect (Anemia, Megaloblastic)*. In: The 5 Minute Veterinary Consult, Tilly, L., and Smith, F., ed., Williams and Wilkins, Media, PA. 1997.
17. **Rebar, A.H.**, *Hemogram Interpretation for Dogs and Cats*, Ralston Purina Company, St. Louis, MO, The Gloyd Group, Inc., 1998.
18. **Rebar, A.H.**, Boon, G.D., and Christian, J.A., *Biochemical Profiling in the Dog and Cat. A Case Oriented Approach*. Ralston Purina Company, St. Louis, MO, The Gloyd Group, Inc. 1999.
19. **Rebar, A.H.**, MacWilliams, P.S., Feldman, B.F., Metzger, F.L., Pollock, R.V.H., Roche, J., *A Guide to Hematology in Dogs and Cats*. Teton NewMedia, Jackson, WY. 2002.
20. Giger, U., **Rebar, A.H.**, Feldman, B.F., *Using White Blood Cell Information More Effectively: A Logical Approach* as part of Hematology Symposium. A Supplement to Compendium on Continuing Education for the Practicing Veterinarian, Vol. 25, No. 9(A), September 2003.
21. Thrall, M.A., Baker, D.C., Campbell, T.W., DeNicola, D., Fettman, M.J., Lassen, E.D., **Rebar, A.H.**, Weiser, G., *Hematology and Clinical Chemistry*, Lippincott Williams & Wilkins, Baltimore, MD, 2004.
22. Thrall, M.A., Baker, D.C., Campbell, T.W., DeNicola, D., Fettman, M.J., Lassen, E.D., **Rebar, A.H.**, Weiser, G., *Clinical Case Presentations for Veterinary Heamtology and Clinical Chemistry*, Lippincott Williams & Wilkins, Baltimore, MD, 2005.

APPENDIX 6.4

GRANT NUMBER: _____

BIOGRAPHICAL SKETCH

Provide the following information for all ***new*** key personnel.
Copy this page for each person.

NAME	POSITION TITLE
Philip J. Bergman, DVM, MS, PhD, DACVIM	Head, Donaldson-Atwood Cancer Clinic

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
University of Arizona - Tucson, AZ		Undergrad	Microbiology
Colorado State University - Fort Collins, CO	DVM, BS	1986-1990	Veterinary Medicine
Kansas State University - Manhattan, KS		1990-1991	Small Animal Intern
Colorado State University - Fort Collins, CO	MS	1991-1994	Oncology Residency
M.D. Anderson Cancer Center - Houston, TX	PhD	1994-1999	Cancer Biology

Professional Positions:

1990-1991	Rotating Small Animal Intern, Kansas State University, Manhattan, KS
1990-1994	Comparative Oncology Residency, Colorado State University, Fort Collins, CO
1994-1999	Cancer Biology Fellow, M.D. Anderson Cancer Center, Houston, TX
1994-1997	Staff Oncologist, Gulf Coast Veterinary Specialists, Houston, TX
1996-1999	American Cancer Society Physician Research Training Fellow, M.D. Anderson Cancer Center, Houston
1998-2000	Chair, ACVIM Forum, Oncology Subspecialty
1999-Present	Head, Donaldson-Atwood Cancer Clinic, The Animal Medical Center, New York, NY
2000-2002	President-Elect, Veterinary Cancer Society
2001-Present	Director, Flaherty Comparative Oncology Laboratory
2002-Present	Adjunct Associate Faculty Member, Memorial Sloan-Kettering Cancer Center & Sloan-Kettering Cancer Institute
2002-2004	President, Veterinary Cancer Society

Honors and Awards:

1989	R. Barry Prynn Memorial Scholarship. *For excellence in neurology.*
1990	AAHA Senior Student Award. *For excellence in small animal medicine and surgery.*
1993	William K. Riddell Memorial Scholarship. *For imminent success & broad impact in the biomedical research field.*
1999	R.E. "Bob" Smith Fellow, Department of Cell Biology, M.D. Anderson Cancer Center. AACR Symposium, Molecular Biology in Clinical Oncology. Invited Participant (Aspen, CO).
1997 & 1998	UT-Houston Graduate School of Biomedical Sciences Travel Award
1996 – 1999	American Cancer Society Physician Research Training Fellowship (PRTA #40) AACR Clinical Trials Symposium. Invited participant (Vail, CO)
2001	Jean Holzworth Keynote Address, Angell Memorial Animal Hospital
2001	Japanese Veterinary Cancer Society Keynote Address
2002	Adjunct Associate Faculty, Memorial Sloan-Kettering Cancer Center & Sloan-Kettering Cancer Institute
2003	World Small Animal Veterinary Association Hill's Award for Excellence in Veterinary Healthcare

Bibliography:

1. **Bergman PJ**, Withrow SJ, Straw RC, et al. Canine Infiltrative Lipoma: 16 Cases (1981-1992). *J Amer Vet Med Assoc* 1994;205:322-324.
2. **Bergman PJ**, Bruyette DS, Coyne BE, et al. Paraneoplastic Clinical Peripheral Neuropathy Associated with Canine Insulinoma. *Prog Vet Neuro* 1994;5:57-62.
3. Ogilvie GK, Moore AS, Chen C, Ciekot PA, Atwater SW, **Bergman PJ**, Walters LM. Toxicoses associated with the administration of mitoxantrone to dogs with malignant tumors: A dose escalation study. *J Amer Vet Med Assoc* 1994;205:570-573.
4. Ogilvie GK, Atwater SW, Ciekot PA, **Bergman PJ**, et al. Incidence of anaphylaxis associated with the intramuscular administration of L-asparaginase to 81 dogs with cancer: 1989-1991. *J Am Anim Hosp Assoc* 1994;30:62-65.
5. **Bergman PJ**, Ogilvie GK. Drug Resistance and Cancer Therapy. *Compend Contin Educ* 1995;17:549-558.
6. Macy DW, **Bergman PJ.** Vaccine-induced sarcomas in cats. *Feline Practice* 1995;23:24-27.
7. Gupta KP, Ward NE, Gravitt KR, **Bergman PJ**, O'Brian CA. Partial Reversal of Multidrug Resistance in Human Breast Cancer Cells by an N-myristoylated Protein Kinase C- Pseudosubstrate Peptide. *J Biol Chem* 1996;271:2102-2111.
8. **Bergman PJ**, MacEwen EG, Kurzman IL, et al. Amputation and Carboplatin for Treatment of Dogs with Osteosarcoma (48 Cases). *J Vet Intern Med* 1996;10:76-81.
9. **Bergman PJ**, Ogilvie GK, Powers BE. Monoclonal Antibody C219 Immunohistochemistry in canine Lymphoma: Predictive Ability and Sequential Analysis. *J Vet Intern Med* 1996;10:354-359.
10. **Bergman PJ**, Gravitt KR, Ward NE, Gupta KP, O'Brian CA. An N-myristoylated Protein Kinase C-Pseudosubstrate Peptide that Partially reverses Multidrug Resistance in Human Breast Cancer Cells is not a P-glycoprotein Substrate. *Cancer Chemotherapy Pharmacology* 1997;40:453-456.
11. McCaw DL, Miller MA, **Bergman PJ**, Withrow SJ, Moore AS, Knapp DW, Fowler D, Johnson JC. Vincristine therapy for mast cell tumors in dogs. *J Vet Intern Med* 1997;11:375-378.
12. **Bergman PJ**, Gravitt KR, Ward NE, Beltran P, Gupta KP, O'Brian CA. Potent induction of human colon cancer cell uptake of chemotherapeutic drugs by N-myristoylated protein kinase C-alpha (PKC-alpha) pseudosubstrate peptides through a P-glycoprotein-independent mechanism. *Inv New Drugs* 1997;15:311-318.
13. **Bergman PJ.** Etiology of feline vaccine-associated sarcomas: history and update. Vaccine-Associated Feline Sarcoma Symposium. *J Amer Vet Med Assoc* 1998;213:1424-1425.
14. **Bergman PJ.** Advances in the Treatment of Feline Vaccine-Associated Sarcomas. *Adv Small Anim Med Surg* 2000.
15. O'Brian CA, Stewart JR, Ward NE, **Bergman PJ**. Regulatory mechanisms governing protein kinase C signaling. *Electr J Pathol Histol* 2000;6:1-10.
16. Rocha TA, Mauldin GN, Patnaik,AK, **Bergman PJ**. Prognostic Factors in Dogs with Urinary Bladder Carcinoma. *J Vet Intern Med* 2000;14:486-490.
17. Kovak JR, Ludwig LL, Noone KE, **Bergman PJ**, et al. The use of thoracoscopy to diagnose pleural effusion of ambiguous etiology. *J Am Vet Med Assoc* 2002;221(7):990-994.
18. Charney SC, **Bergman PJ**, Hohenhaus AE, McKnight JA. Risk factors for sterile hemorrhagic cystitis in dogs with lymphoma receiving cyclophosphamide with or without concurrent administration of furosemide: 216 cases (1990-1996). J Am Vet Med Assoc 2003;222(10):1388-1393.
19. **Bergman PJ**, McKnight J, Novosad A, Charney S, Farrelly J, Craft D, Sadelain M, Wulderk M, Jeffers Y, Hohenhaus AE, Segal N, Gregor P, Engelhorn M, Riviere I, Houghton AN, Wolchok JD. Long term survival of dogs with advanced malignant melanoma following DNA vaccination with xenogeneic human tyrosinase: A phase I trial. Clin Cancer Res 2003;9:1284-1290.
20. **Bergman PJ**. Clinical techniques in small animal molecular oncology. Clin Tech Small Anim Pract 2003;18:88-91.
21. Simpson AM, Ludwig LL, Newman SJ, **Bergman PJ**, Hottinger HA, Patnaik AK. Canine cutaneous mast cell tumors: A prospective study of surgical margins. *J Am Vet Med Assoc* 2004;224(2):236-240.
22. Farrelly J, Denman DL, Hohenhaus AE, Patnaik AK, **Bergman PJ**. Hypofractionated radiation therapy of oral melanoma in five cats. *Vet Radiol Ultras* 2004;45(1):91-94.
23. Newman SJ, **Bergman PJ**, Williams B, Scase T, Craft D. Characterization of Spindle Cell Component of Ferret (Mustela putorius furo) Adrenal Cortical Neoplasms – Correlation to Clinical Parameters and Prognosis. *Comp & Vet Oncol* 2004;2(3):113-124.

24. Patnaik AK, Newman SJ, Scase T, Erlandson RA, Antonescu C, **Bergman PJ**. Canine hepatic neuroendocrine carcinoma: An immunohistochemical study of 10 cases. *Vet Pathol* 2005, in press.
25. Jakubiak MJ, Zenger E, Siedlecki CT, Matteucci ML, Bruskiewicz KA, Rohn DA, **Bergman PJ**. Laryngeal, laryngotracheal and tracheal masses in cats: 27 cases (1998-2003). *J Am Anim Hosp Assoc* 2005, in press.
26. Winston J, Craft DM, Scase TJ, **Bergman PJ**. Immunohistochemical detection of Her-2/neu expression in spontaneous feline mammary tumors. *Vet Comp Oncol* 2005, in press.
27. McAbee KP, Ludwig L, Newman S, **Bergman PJ**. Cutaneous Hemangiosarcoma in 18 cats. *J Am Anim Hosp Assoc* 2005, in press.
28. Allenspach K, Grone A, Doherr MG, **Bergman PJ**, Gaschen F. P-glycoprotein expression in lamina propria lymphocytes of duodenal biopsies in dogs with inflammatory bowel disease. *J Vet Intern Med* 2005, submitted.
29. Charney SC, **Bergman PJ**, McKnight JA, Farrelly J, Novosad CA, Leibman NF, Camps-Palau MA. Evaluation of intracavitary mitoxantrone and carboplatin for treatment of carcinomatosis, sarcomatosis, and mesothelioma, with or without malignant effusions: A retrospective analysis of 12 cases (1997-2002). *Vet Comp Oncol* 2004, in press.
30. Novosad CA, **Bergman PJ**, O'Brien M, Charney SC, et al (VCOG). Retrospective evaluation of adjuvant chemotherapy for the treatment of feline mammary gland adenocarcinoma. *JAVMA* 2004, submitted.

APPENDIX 6.5

Dr. Ronald D. Schultz
Professor and Chair
Department of Pathobiological Sciences
School of Veterinary Medicine
University of Wisconsin-Madison

EDUCATION: North Penn High School, Lansdale, Pennsylvania, 1962
The Pennsylvania State University
BS (1966), MS (1967), PhD (1970)
Major - Microbiology/Immunology
Minors - Biochemistry and Veterinary Pathology

MILITARY SERVICE: Captain, Medical Service Corps, U.S. Army Reserve, 1966-1975

POSITIONS HELD:

1982 - present	Professor (Tenured) and Chair, Department of Pathobiological Sciences, School of Veterinary Medicine. Professor (Joint Appointment), Department of Medical Microbiology and Immunology, School of Medicine. Professor (Affiliate Appointment), Department of Animal Health and Biomedical Sciences, College of Agriculture and Life Sciences. Professor, Environmental Toxicology Center, University of Wisconsin, Madison, Wisconsin.
1978 - 1982	Professor of Immunology (Tenured) and member of Graduate Faculty, Department of Microbiology and Animal Health Research Unit, School of Veterinary Medicine, Auburn University, Auburn, Alabama.
1977 - 1978	Associate Professor of Immunology (Tenured), James A. Baker Institute for Animal Health Research (Veterinary Virus Research Institute), Cornell University, Ithaca, New York.
1973 - 1978	Assistant Professor of Immunology and member of Graduate Faculty, James A. Baker Institute for Animal Health, Cornell University.
1972 - 1978	Associate Director, Human Health Services Clinical Laboratory, Department of Health Services, Cornell University, Ithaca, NY.
1971 - 1973	Research Associate, Department of Microbiology, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York.

HONORS & MEMBERSHIPS:

First President American Association of Veterinary Immunologists
Honorary Diplomate of American College of Veterinary Microbiologists
Distinguished Veterinary Immunologist Award, American Association of Veterinary Immunologists, 1988
Walter F. Renk Distinguished Professor Award, Faculty, School of Veterinary Medicine, University of Wisconsin-Madison, 1989.
President of Conference of Research Workers in Animal Diseases, 1994.
Served on all School of Veterinary Medicine Committees and Numerous Campus Committees
Served on Review Committees for USDA, FDA, NIH and private foundations
Editorial Board Member for various journals

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RESEARCH INTERESTS

-Developmental aspects of immunology
-Immunopathology associated with viral diseases
-Immunomodulators
-Clinical immunology and virology
-Vaccinology
- Zoonotic diseases and foreign animal diseases

Trained approximately 50 graduate students and postdoctoral fellows

Published approximately 200 articles in peer reviewed journals, edited several books and hold a number of patents

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APPENDIX 6.6

LAWRENCE T. GLICKMAN

Biographical Sketch

Dr. Glickman is on the faculty of the Purdue University School of Veterinary Medicine. In addition to a degree in veterinary medicine, he has a doctoral degree in public health and a master's degree in physiology. He is board certified by the American College of Epidemiology. He served as Chairman of a committee of the National Academy of Sciences that authored a monograph titled *Animals as Sentinels of Environmental Health Hazards*. He has published over 275 journal articles, book chapters, and monographs, related to human and animal health. He studies relationships between humans, animals, and the environment. Dr. Glickman has received grants and contracts from federal agencies including the National Institutes of Health, the Centers for Disease Control and Prevention, the Food and Drug Administration, the U.S. Department of Education, and the U.S. Department of Agriculture, and from private foundations, breed clubs, and industry. He has been recognized for contributions to human and animal health with honors including the Award of Recognition in Veterinary Public Health and Preventive Medicine in 1988 from the Teachers of Veterinary Preventive Medicine and Public Health, the Pfizer Award for Research Excellence in 1997, an award from the University of Pittsburgh Graduate School of Public Health in 1999 as one of the 50 top contributors to public health over the past 50 years, an Alumni Award of Merit in 2002 from the University of Pennsylvania School of Veterinary Medicine for advancing animal health, and the 2003 AKC Award for Excellence in Canine Research. He has trained more than 20 PhD students in epidemiology and chairs the Section of Clinical Epidemiology at Purdue University. Dr. Glickman directed the largest prospective companion animal health study to date, involving nearly 2000 pet dogs that were followed for five years to identify the causes of gastric torsion. His current research utilizes computerized veterinary medical records to detect biological, chemical, and physical hazards, resulting from acts of terrorism and to measure the incidence of adverse health effects associated with veterinary vaccines and pharmaceuticals. Dr. Glickman recently published the results of two studies showing that lawn herbicides are a probable cause of bladder cancer in dogs and chemicals in food can linings may be responsible for the current epidemic of hyperthyroidism in cats. He developed a simple method called the *Vaccinometer* to help veterinarians weigh the risks versus benefits of vaccines for individual patients. He is a strong advocate for the evidence-based approach to veterinary medical practice and preventive medicine.

Current Position	Professor of Epidemiology & Public Health Head, Section of Cli nical Epidemiology
Office Address	Department of Veterinary Pathobiology School of Veterinary Medicine Purdue University West Lafayette, IN 47907 -1243

Education

B.A.	1964 - Biology, State University New York at Binghamton
M.A.	1966 - Physiology, State University New York at Binghamton
V.M.D.	1972 - University Pennsylvania, School Veterinary Medicine,
M.P.H.	1975 - Epidemiology and Infectious Diseases, University Pittsburgh, Graduate School of Public Health
Dr.PH.	1977 - Epidemiology and Public Health, University Pittsburgh, Graduate School of Public Health

Postgraduate Training

1975 -1976	N.I.H. Postdoctoral Fellow in Cardiovascular Disease Epidemiology, Graduate S chool Public Health, University Pittsburgh
1976 -1977	Research Assistant, Department of Epidemiology, Graduate School Public Health, University Pittsburgh

Military Service

1960 -1966	United States Army National Guard, (Honorable Discharge)

Academic Appointments

1977-1980	Assistant Professor of Epidemiology and Preventive Medicine, Department of Preventive Medicine, and Head, Division of Epidemiology, Diagnostic Laboratory, New York State College of Veterinary Medicine, Cornell University
1980-1987	Associate Professor of Epidemiology and Chief, Section of Epidemiology and Public Health, Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania (Tenure granted July 1, 1983)
1980-1985	Faculty in Graduate Group of Epidemiology, University of Pennsylvania
1981-1988	Faculty in Graduate Group of Comparative Medical Sciences, University of Pennsylvania
1982-1988	Faculty in Graduate Group of Parasitology, University of Pennsylvania
1985-1988	Adjunct Professor of Epidemiology, Section of General Medicine, Hospital of the University of Pennsylvania
1987-1988	Professor of Epidemiology and Head, Section of Epidemiology and Public Health, Department of Clinical Studies, School of Veterinary Medicine University of Pennsylvania
1988-1993	Head, Department of Veterinary Pathobiology, Purdue University, School of Veterinary Medicine
1988-1993	Head, Graduate Program in Veterinary Pathobiology with subspecialties in pathology, epidemiology and public health, microbiology, parasitology, and immunology, Purdue University Graduate School.
1988-present	Professor of Epidemiology and Public Health and Head of Section of Clinical Epidemiology, Department of Veterinary Pathobiology, Purdue University, School of Veterinary Medicine
1991-present	Adjunct Professor of Epidemiology, Indiana University School of Medicine, Indianapolis, IN
1991-present	Founding Member, Purdue University Interdisciplinary Graduate Program in Nutrition

Hospital and Administrative Appointments

1966-1967	Research Scientist, Research and Drug Development, Endo Laboratories, Garden City, New York
1972-1974	Practicing Veterinarian, Trooper Veterinary Hospital, Norristown, PA
1976-1977	Assistant Director, Laboratory Animal Care Facility, School of Medicine, University of Pittsburgh (Major responsibility for dogs, cats, and non-human primates)

Specialty Board Certification

1982	Fellow of the American College of Epidemiology

Awards, Honors, and Membership in Honorary Societies

- 1972- Borden Award for highest academic average in veterinary school
- 1972- J.B. Lippincott Prize for academic achievement in veterinary school
- 1972- Leonard Pearson Prize for Advancement of Veterinary Science and Research in Practice, Education and in Civilization
- 1972- 1930 Class Prize-for highest average in surgery
- 1983- Ralston Purina Small Animal Research Award
- 1987- Veterinary Student Government Award for Excellence in Teaching, University of Pennsylvania
- 1988- Award of Recognition in Veterinary Public Health and Preventive Medicine, Teachers of Veterinary Preventive Medicine and Public Health
- 1989- Delta Omega Society, for outstanding attainment in public health
- 1989- Sigma Xi National Scientific Research Honor Society
- 1991- Merck AGVET Award for Creativity in Veterinary Education
- 1991- National Society of Phi Zeta, Honor Society of Veterinary Medicine
- 1997- Pfizer National Award for Research Excellence
- 1999- University of Pittsburgh Public Health Award, Top 50 contributors to public health in past 50 years
- 2002- Alumni Award of Merit, University of Pennsylvania School of Veterinary Medicine
- 2003- American Kennel Club-American Veterinary Medical Association, Excellence in Canine Research

Memberships in Professional and Scientific Societies

- American Academy Veterinary Disaster Medicine
- American Academy Veterinary Nutrition
- American College Epidemiology (Fellow status)
- American Public Health Association (Chairman, Membership Committee, Veterinary Public Health Section, 1982-1986)
- American Veterinary Medical Association
- Association of Teachers Veterinary Public Health and Preventive Medicine
- Association of Veterinary Medical Educators
- National Association State Public Health Veterinarians
- Society for Epidemiologic Research
- Society of Toxicological Pathologists (Associate Member)
- Teachers of Veterinary Preventive Medicine and Public Health (Executive Board, 1987-1988)

Peer Reviewed Publications

Approximately 300 publications in medical journals, textbooks, and monographs
A detailed list is available upon request